AD2

163

U. S. DEPARTMENT OF AGRICULTURE.

BUREAU OF PLANT INDUSTRY—BULLETIN NO. 213.

B. T. GALLOWAY, *Chief of Bureau.*

CROWN-GALL OF PLANTS: ITS CAUSE AND REMEDY.

BY

ERWIN F. SMITH, Pathologist in Charge of Laboratory of Plant Pathology,

NELLIE A. BROWN, Scientific Assistant,

AND

C. O. TOWNSEND, formerly Pathologist in Charge of Sugar-Beet Investigations.

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119. The Mulberry and Other Silkworm Food Plants. 1907.
120. Production of Easter Lily Bulbs in the United States. 1908.

213 [Continued on page 3 of cover.]

U. S. DEPARTMENT OF AGRICULTURE.

BUREAU OF PLANT INDUSTRY—BULLETIN NO. 213.

B. T. GALLOWAY, *Chief of Bureau.*

CROWN-GALL OF PLANTS: ITS CAUSE AND REMEDY.

BY

ERWIN F. SMITH, PATHOLOGIST IN CHARGE OF LABORATORY OF PLANT PATHOLOGY,

NELLIE A. BROWN, SCIENTIFIC ASSISTANT,

AND

C. O. TOWNSEND, FORMERLY PATHOLOGIST IN CHARGE OF SUGAR-BEET INVESTIGATIONS.

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213

LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Plant Industry,
Office of the Chief,
Washington, D. C., February 6, 1911.

Sir: I have the honor to transmit herewith and to recommend for publication as Bulletin No. 213 of the special series of this Bureau the accompanying technical paper by Dr. Erwin F. Smith, Miss Nellie A. Brown, and Dr. C. O. Townsend, entitled "Crown-Gall of Plants: Its Cause and Remedy."

This paper deals with an infectious disease of fruit trees and many other economic plants which, because of its infectious character, has spread to many parts of the United States. It is known to occur also in Europe and Africa.

The importance of this disease is evident from the frequency of appearance of references to it and the amount of literature already published regarding it. Various theories have been advanced as to its cause, many of these by men of high standing in pathological work, but none have been able to establish their theories conclusively. The disease has been ascribed to frost injuries, to fungi, to slime molds, and to various small animals found infesting the older galls. By practical orchardists and by most pathologists crown-gall is generally regarded as a dangerous and destructive disease; by some others it has been considered nonparasitic and of little economic importance.

The investigations here reported upon have covered a period of six years, during which the nature of the disease has been determined, its cause discovered, and its broadly infectious character established through hundreds of carefully conducted experiments. Its ready communicability by inoculation from plants of one natural family to another is thoroughly established and indicates its importance to the farmer and the horticulturist.

As the problems involved are varied and important, with very practical bearing on the production of a wide range of crop plants, it is important that the well-established evidence accumulated in

these investigations be presented in full. With the cause of the disease once generally accepted as established, the practical questions relating to its control will be much simplified.

The illustrations submitted are essential to a proper understanding of the text.

Respectfully,

WM. A. TAYLOR,
Acting Chief of Bureau.

Hon. JAMES WILSON
Secretary of Agriculture.

213

CONTENTS.

ILLUSTRATIONS.

PLATES.

213

TEXT FIGURES.

B. P. I.—650.

CROWN-GALL OF PLANTS: ITS CAUSE AND REMEDY.

HISTORY IN BRIEF.

EUROPE.

This disease has been known in Europe on various plants for many years (50 or more in literature) and has been generally ascribed to frosts or to mechanical injuries (Goethe, Viala, Sorauer, Briem, Prillieux, and others); but to bacteria by Corvo? (Phylloxera of grape) 1885; Cuboni (grape) 1889; Comes (grape) 1892; Cavara (grape) 1897 and 1900, (peach? and juniper?) 1898; Scalia (rose) 1903; and Brizi (poplar) 1907.

Cavara was the first to demonstrate the bacterial nature of the disease (on vines) by means of inoculations from pure cultures. His studies, as well as those of other writers of southern Europe, having been generally overlooked in this country, it appears to be worth while to summarize the leading French and Italian papers somewhat carefully, in so far as they have ascribed this disease to bacteria.

The labors of Corvo and Cuboni being early, they are not bacteriological in any modern meaning of that term. Their views as to the cause of the disease appear to the writers to have been happy guesses based on the resemblance of the tumors of the grape (rogna) to the tubercle of the olive, then much talked about, rather than opinions based on convincing proofs, for the reason that the bacteria seen by them appear to have been common saprophytes lodged in cracks and crevices of the dead parts of the older galls, quite after the manner of the bacterial occupation of the olive tubercle, whereas the actual gall-forming organism in this case, as Cavara first pointed out, occurs in small numbers scattered through sound-looking tissues, and is very difficult to see even when stained. This will be better understood after reading the following summary:

In 1885 Corvo published a note (C. R. des Sé. de l'Acad. des Sci., Paris, T. 101, p. 528) in which he maintained that there is no such disease as Phylloxera of the vine, strictly speaking, but only a tuberculosis due to bacteria which are transmitted by the insects, but

213

which may also induce infections independently of them. Corvo's statements do not appear to have received any attention from students of Phylloxera in France or elsewhere, and his bacteriological technic is wholly negligible.[a] The organism is supposed to be a bacillus. It was obtained [sic] by putting the brown slime and fragments of tissue into flasks of vine juice diluted with distilled water, no mention being made of any surface sterilization of the fragments or of any preliminary sterilization of the culture fluid. The fluid clouded after a few days, and the infections were then obtained by plunging split vine shoots into this yellowish rotting fluid; again it would appear without surface disinfection. The results obtained appear to have been limited to a yellow-brown stain in the tissues, which is said to be the striking characteristic of the disease. In this stained portion, naturally, organisms were found.

In 1889 Cuboni published a note on the subject in the Atti della Reale Accademia dei Lincei (Rendiconti), Rome, vol. 5, p. 571. He reported finding an abundance of bacteria in the tubercles of the vine, but they appear to have been limited to dead portions. The important part of his paper is included in the following citation:

> The examination of microscopic sections made from scabby shoots collected the previous year and preserved in alcohol has demonstrated that in fact in all the tubercles are to be found masses of bacteria wholly identical with those which are to be observed in the tubercles of the olive. These bacteria are united into zooglœæ by a mucilaginous substance insoluble in alcohol, filling the small canals or lacunæ which are found scattered irregularly throughout the tubercle. The dimensions of the bacteria vary from 1 to 1.5 μ and are about 0.3 μ broad. In the unstained sections placed in glycerine these bacteria are strongly refringent to light; treated with methyl violet they stain quite feebly.
>
> The cells which surround the lacunæ occupied by the bacteria are dead and in great part corroded. The walls of the cells remaining are of a yellowish-brown color, so that the naked eye is able to recognize in a section the nodules and the little canals where the colonies of the bacteria occur. Surrounding the lacunæ, i. e., beyond the zone of dead cells, are found parenchyma cells full of protoplasm with nuclei, many cells filled with starch granules, and also here and there strata of suberized cells alternating with strands of large bast fibers, and finally the woody elements, especially tracheids, contorted in an odd fashion and the whole arranged in an irregular manner, so that it is often difficult to orient one's self as to the genesis of the various elements.

No mention is made of any cultures or inoculations.

In 1889 Trevisan published for the above-indicated bacteria the name *Bacillus ampelopsorae*, drawing his account entirely from the notes by Cuboni and Savastano's reference to Corvo. At least there is no evidence that Trevisan himself took the trouble to make even a cursory microscopic examination, his habit being to name everything left unnamed by others, without troubling himself to

[a] Petri makes no mention of Corvo, but states (Annales Mycologici, June, 1909) that in the galls of Phylloxera he found scarcely any bacteria: "The bacteria were very rare, and never have I been able to isolate from the galls the *Bacillus vitis*."

make studies. Inasmuch as this name was given without inoculations or study of cultures, and as various bacteria occurring in the tumors are indistinguishable microscopically, and the saprophytes often more abundant than the parasite, and particularly as the parasitic organism does not, so far as we know, congregate in zooglœæ masses in cracks or lacunæ of the dead tissue in the manner described for this organism, his name is not here used. His description in full is as follows:

Bacillus ampelopsorae Trev. in add. ad Gen. pag. 36, Batterio della rogna della vite Cuboni in Rendiconti Accad. d. Lincei, Ser. IV, 1889, Vol. V., p. 571, Bacterie de la tuberculose de la Vigne Andrade Corvo in Savast. Compt. Rend. Paris, 1886.—Baculis cylindraceis, 1 to $1.5 \times 0.3\,\mu$, in colonias canaliculos lacunasque tumefactionis implentes congregatis.

Hab. in tumoribus Vitis.—Dilute coloratur per colorem violaceum methylicum. *B. oleae* analogus.

In 1897 Cavara described the tuberculosis or rogna of the vine from material obtained near Venice (Stazioni Sperimentali Agrarie Italiane, Modena, vol. 30, p. 483) and partially removed the subject from the region of uncertainties by making pure cultures of the right organism and successful inoculations therefrom. His experiments, however, were so few in number that they did not convince anyone and were generally overlooked. He had published a brief preliminary note in 1895, but the following citations are made from the later and fuller article:

The attacked plant presents the following characters: Rachitic development of the leaves; color of the leaf blade greenish yellow. The leaf blades are bent on the margins and acquire a waxy aspect quite analagous to leaves of the peach attacked by *Exoascus deformans*. The branches of the year may be of a yellowish color and enlarged, and, abstraction made of the tubercular part which forms at the nodes, assume a size sometimes double or triple the normal size. The tubercules on the young shoots appear first at the nodes, in correspondence with which the periderm is lifted up in tense bridles; but then they extend to the internodes, the bark of which cracks open in a longitudinal direction. The old shoots show ample crevices, the margins of which are lined with close-set and small tubercles which, as they become old, decompose and show a browned, decayed surface. * * *

I cultivated this bacterium in gelatin prepared with green shoots of the vine, and the Petri-dish poured plates gave circular, flat colonies, mother-of-pearl color, which did not liquefy the surrounding gelatin. In tube culture, transferring from the plate culture by means of the needle, the infections gave a colony pure mother-of-pearl color with disappearing edges, sunken a little in the center while assuming a granular aspect along the line of the stab. The bacterium did not liquefy the gelatin, and behaved like an aerobe. In agar agar it was cultivated also very well, but the development was slower. Stained with methylene blue, the organisms obtained from various cultures and observed under the microscope showed a cylindric form with the extremities rounded, without any refringent particles, and having a form rather of a bacterium than of a bacillus, the dimensions being 1.5 to $2 \times 0.5\,\mu$.[a]

With cultural material many times renewed by transfer, I made inoculations in the Botanic Garden of Pavia, where in the garden plot of the Ampelopsidæ were

[a] Compare our own measurements, pp. 105, 128.

planted various species of vines, some of which were American, others Asiatic, along with two varieties of *Vitis vinifera.* There were six shoots which, by means of T-shaped incisions made after the necessary sterilization, were inoculated either in the lower internodes of shoots of the year or in upper internodes, while as many were simply incised without inoculation of the virus, for reasons of control. All were then wound with taffeta and with paper and tied with thread at the point of incision.

This inoculation experiment was made in July, therefore in a stage too advanced perhaps to have hope of any success whatsoever. Weeks and months passed away without there being shown in any of the vines any traces whatsoever of localized and diffused hypertrophy. So I lost all hope and ceased to visit them any more.

Toward the end of the winter the head gardener of the Botanic Garden, Signor Giacomo Traverso, in pruning the vines of the above-mentioned garden plat, was surprised by certain nodules which one of the European vines showed, and as he had assisted me in the experiment, came to inform me in the laboratory with a specimen which he had cut off, and which bore just two of the characteristic tubercles in correspondence to the node and with the periderm raised up in bridles in a manner exactly identical with that which had been observed on the vine shoots collected at Udine.

Of the two varieties of European grape, one was inoculated in four of its branches, the other left with simple incisions for control. All of the four inoculated branches gave tubercles, not of large dimensions, it is true (1 to 1.5 cm. in diameter); but, I repeat, of the same form and structure of those of the vine from Udine. The foreign vines were not attacked.

The microscopic examination of the shoots bearing the tubercles likewise revealed bacteria scattered in the vessels and in the tissues of the bark of the small tubercles, and I was able to establish that they do not form distinct foci as in the case of the olive and the Aleppo pine, but are found scattered here and there in the various tissues.

In order better to ascertain that bacteria of the rogna were really in question I made some cultures with material of the same origin, infected in the Botanic Garden, and obtained both on plates and in test-tube cultures the same mother-of-pearl color colonies, and the same bacteria which had served for the inoculation experiment.[a]

In 1890, Cavara published a second paper on the subject, but this is rather of a popular nature and adds nothing to the preceding paper, except notes on the occurrence of the disease in Sardinia, and a very good lithographic plate in colors, showing vine shoots bearing the tubercles, which are like those occurring in the United States.

Although Cavara's account is meager, there is little doubt in the light of our own experiments that he had the right organism and reproduced the disease with it as he says he did.

In the same paper (Staz. Sper. Ital., 1897, vol. 30, p. 504) Cavara describes a tuberculosis of the peach occurring frequently on young shoots in a garden at Pavia, and attributes this also to bacteria, which he states he cultivated out. He made no inoculations in this case and his account of the disease renders it uncertain whether he had to do with pathological formations identical with those occurring on the peach in this country and studied by us. Our peach disease occurs more commonly on crown and roots than on branches, but this may be only a matter of environment. Doctor Farneti, who

[a] Had Dr. Cavara obtained additional infections with these isolated bacteria he would have completed his proof.

furnished the material to Doctor Cavara, was good enough to collect similar material for the senior writer in the summer of 1906, but unfortunately poured plates were not made therefrom, and we can not come to any definite conclusion from the appearance of the specimens. In any event, the dendritic, spore-bearing, liquefying organism which Doctor Cavara isolated and described from these tumors under the name of *Clostridium Persicae tuberculosis* is quite distinct from the one causing the crown-gall of the peach in this country, and therefore need not be taken into consideration here.

In 1898 Cavara (Bull. d. Soc. Bot. Ital., p. 241) also described a tumor from juniper, which he attributed to bacteria. Two species were isolated, a micrococcus liquefying gelatin slowly, and a rod which grew as a white mass and rapidly liquefied gelatin. In this case he made inoculations, but not on the same species of juniper, and did not obtain any positive results with either organism. In 1904 Baccarini (Nuovo Giornale Bot. It., p. 49) and in 1910 Severini (Annali di Botanica, p. 253) claimed this tumor to be due to a Ceratostoma.

In 1903 Doctor Scalia, in Sicily, described a tumor occurring on old stems of the rose near the surface of the earth, but also frequently higher up. His paper deals principally with signs of the disease and the anatomy of the healthy and diseased parts. He does not appear to have made any cultures or inoculations, but on the strength of his microscopic examinations he named the organism *Bacillus rosarum*. He states that he discovered it in very thin sections in the interior cells of the hypertrophied tissue and in the brown gum. The bacteria were numerous in the form of small rods with rounded extremities, measuring 1 to 1.5 μ by 0.2 to 0.3 μ. They were stained with methyl violet by putting thin sections into a drop of water (which may have been sterile, although he does not say so or mention any checks), removing the sections after a short time, allowing the drop to dry, and then fixing and staining what remained upon the glass. The diameter of this organism conforms to Cuboni's measurements and is less than that of *Bacterium tumefaciens*.

It is impossible to be quite certain that the disease described by Scalia is identical with the crown-gall of the rose as it occurs in this country, and, of course, without proofs from inoculations or any description of the cultures, a name such as he has given is worthless for scientific purposes and should be regarded as a *nomen nudum*. Owing to tbe soft nature of the rose gall and the ease with which it disintegrates one might expect to find almost any saprophyte in the brown gum.

Von Thümen in Austria attributed the tuberculosis of the grape to a fungus, Fusisporium, but without offering any convincing

proofs. In the same way Laubert and Köck in Austria have ascribed the rose canker to Coniothyrium (1905).

Stoklasa in Bohemia ascribed the beet gall to nematodes (Tylenchus) and Bubák in Austria to mites (Histiostoma), but nematodes and mites are present in true crown gall, so far as we have observed, only after decay sets in. Possibly Stoklasa had to do with nematode galls which also occur on the beet. (Pl. IV, fig. 1.)

This disease, as it appears on the vine, is known in France as Broussins, in Italy as Rogna, and in Germany as Krebs or Grind. Viala also gives various other names, as Exostoses, Exostoses fongoïdes, Fongosités, Raude, Kropf, Schorf, Ausschlag, Mauke, Hanab, Tubercoli, Malattia dei tubercoli, etc. The gall on the sugar beet is known in Germany as the Wurzelkropf.

This brings the European history of crown-galls, so far as ascribed to bacteria, down to the appearance of the first paper on the subject by the senior writers of this Bulletin in April, 1907 (Science), a translation of which, with some additions, was published in Germany. (Centralb. für Bakt. 2 Abt., Bd. 20, December, 1907, p. 89.)

In 1907, Ugo Brizi, of Milan, described and figured a tumor of poplar, ascribed the disease to bacteria, claimed infections with pure cultures, and named and described a yellow schizomycete (*Bacillus populi*) said to be the cause of the galls (Atti Congreso Naturalisti Italiani, Milan, June, 1907). The Congress was held in September, 1906, but the paper was not published until the following summer.

Brizi figures one infection only and gives no details concerning his inoculation experiments, i. e., where they were conducted; how many failed; how many checks were held; and whether any of the latter contracted the disease. His experiments are probably of the same sort as Professor Toumey's, where one known organism was introduced and another unknown (and unsuspected) organism actually caused the disease.

The *Bacillus populi* of Brizi, which is probably one of the yellow saprophytes common in crown-galls, may be distinguished readily from the organism described in this bulletin by the following characteristics, which are summarized from his paper:

Yellow growth on culture media (agar, gelatin, potato, sugar beet, etc.); production of spores, which are generally in one end, which is swollen and refringent; rapid production of indol (24 hours at 30° C.); rapid coagulation of milk (12 hours at 25° C.) and re-solution of the curd; rapid growth in weakly acid beef broth at 25° C., i. e., clouding after a few hours. Motility occurs, but he did not succeed in demonstrating flagella by means of stains. Inasmuch as he has lost his cultures of this organism (letter of Brizi to Král, Sept. 2, 1910), we were unable to obtain it for comparison.

UNITED STATES.

Most of the experimental work on the disease has been performed in the United States. References in literature begin about the year 1892, but undoubtedly the disease has been present for a long time. The literature is so well known and so easily accessible that it is not necessary to abstract it at any length. The most recent papers are by Dr. George G. Hedgcock, of the Bureau of Plant Industry (Bulletins 183 and 186), and he has given therein a rather full bibliography.

The infectious nature of the peach gall was rendered certain several years ago by a number of experiment station workers who obtained the growths on young trees either by planting them in the vicinity of diseased ones, by mincing the galls and distributing the fragments in the sand or earth near sound trees, or by grafting. Thus Thaxter in Connecticut (1891 or prior), Halsted in New Jersey (1897), Selby in Ohio (1898). Toumey in Arizona (1900) proved in the same ways the infectious nature of the almond gall.

As the result of his experiments on almonds Toumey concluded the disease to be due to a slime mold described by him as *Dendrophagus globosus*, but this statement is not sufficiently supported by infection experiments and is regarded by the writers as wholly erroneous. Toumey made only a few inoculations and none with indubitably pure material, i. e., his inoculating material oozed from the cut surface of galls which undoubtedly contained the bacteria here described. It was never grown in pure cultures. Of his 10 inoculations 3 only yielded galls.

Hedgcock subsequently cross-grafted fragments of galls successfully on some fruit trees and unsuccessfully on others. His general conclusions, however, have differed so materially in his various papers that the reader is referred to his texts.

Under the name of necrosis of the grapevine (Cornell Univ. Bull. No. 263, Feb., 1909), Reddick figured this disease and ascribed it to *Fusicoccum viticolum* n. sp., but on insufficient evidence, as he has since admitted (2d Meeting Am. Phytopath. Soc.).

This disease, which is commonly known as crown-gall, occurs, on one plant or another, in all parts of the United States. Toumey's inquiries in 1900 showed it present in 22 States, to which all the others may now be added.

SOUTH AMERICA.

In Chile, according to Delacroix, this disease as it occurs on the vine has been ascribed by Lataste to the root coccid, *Margarodes vitium*.[a]

Solano has reported the disease as occurring on the grapevine in Peru (1910).

[a] Possibly two diseases are confused. The woolly aphis (Schizoneura) induces small galls on stems and roots of the apple and those on the roots have been confused with crown galls.

SOUTH AFRICA.

According to Dr. Thomas F. Dreyer, of Cape Town (oral communication), crown-gall occurs in Cape Colony on pear trees both in the nursery and in the orchard, and large swellings of some sort also occur on the limbs of apple trees.

According to I. B. Pole Evans, plant pathologist at Pretoria (oral communication), galls of this general character are common in South Africa on rose, peach, willow, etc., appearing on the parts above ground, especially after hailstorms, which are of frequent occurrence.

Since these paragraphs were written we have received from Charles P. Lounsbury, Government entomologist for Cape Colony, an account of these galls (Agricultural Journal, April, 1910) entitled "Giant twig-gall of willow, poplar, peach, apple, and other trees," in which he states that the gall is most common on willow (*Salix babylonica*). The poplar (*Populus alba*), peach, apple, apricot, pear, and rose are said to be attacked also. The largest gall seen by Mr. Lounsbury on the willow was 5 inches in length by 3.5 inches in diameter. He says: "Much larger galls than this are said to occur, but ones under 3 inches in length are far more numerous." They are said to be very abundant on the branches of the willow and to injure it seriously, killing the branches beyond the gall. It has been suggested by some that the galls begin in wounds made by hail, and by others that they start from insect punctures. The disease occurs also in the Transvaal. These galls have been investigated by various experts (Mally, MacOwan, Pole Evans, Lounsbury), but no conclusion is reached as to their cause, except that Mr. Lounsbury says that Mr. Pole Evans has discovered that a very common knot "which occurs throughout the land on quince trees" is "associated with a particular fungus."

Willow galls received from Mr. Lounsbury are reported on later in this bulletin (p. 94). Mr. Lounsbury's figures strongly suggest crown-gall, but in conclusion he says: "It seems improbable that it [the American disease] is identical with the South African trouble under discussion."

We have, however, produced the disease on willow with a schizomycete isolated and subcultured from one of his galls.

EARLIER STUDIES IN THE DEPARTMENT OF AGRICULTURE.

The senior writer's first acquaintance with this disease (as it occurs on peach trees) was in 1892 (Journal of Mycology, vol. 7, p. 378). This was the first work on the disease in the Department of Agriculture. In 1893 he spent about six months on the crown-gall of peach, making microscopical studies and cultures with material received from various places in California, and also with some

from Georgia. He did not then have bacteria in mind, but rather plasmodia and fungi, especially the latter, an effort being made to connect the growths with certain roundish brown chlamydospores found very abundantly in some of the galls. Nothing was published on the subject, because the conclusion was finally reached that neither plasmodia nor fungi were the cause of the disease.

Thereafter none of us (the writers) did anything with the subject of crown-gall for a period of 10 years—i. e., until 1904.

In the interim other workers in the Bureau of Plant Industry took up the subject—i. e., Waite, O'Gara, von Schrenk, and Hedgcock, but without discovering the cause. It may also be added that in beginning work on the daisy gall the writers had no idea that it would reopen the whole subject and lead in all sorts of directions.

STUDIES DETAILED IN THIS BULLETIN.

DISCOVERY OF THE BACTERIA, FIRST ISOLATIONS, AND INOCULATIONS.

In February, 1904, the Bureau of Plant Industry received a number of marguerites or Paris daisy plants (*Chrysanthemum frutescens*), both white and yellow varieties, all of which were affected with gall-like growths on various parts of the stems and leaves. These plants were sent in by one of the large commercial daisy growers in New Jersey, and were accompanied with the statement that both old and young plants were attacked, but that the older ones were more seriously affected than the younger ones. The further statement was made that the disease appeared on the plants in the open in summer and in the greenhouse in winter, and that the galls appeared on stems and leaves without any apparent cause. The galls received varied in size from one centimeter to several centimeters in diameter. The smaller and younger galls were green in color, nearly smooth in appearance, and soft and spongy to the touch. As the galls became older they increased in size and darkened in color externally until they were distinctly brown, the surfaces were rough (corky), sometimes convoluted, and they were firm and hard. All gradations were noticeable, so that regardless of the unlike appearance of the different galls, it was evident that they were all of the same origin. New galls appeared from time to time after setting out the plants in our hothouse.

The various conditions under which the galls formed excluded the possibility of their being due to insect injuries. A careful examination of the galls for fungi resulted in none being found in the interior of the tissues, and only one was found on the surface—a Macrosporium—which occurred on a portion of the knots and which had

every appearance of being a saprophyte. Moreover, when the galls were placed under favorable conditions for the development of fungi which might possibly have been overlooked in the microscopic examination, no fungus appeared.

Bacteria in the interior of the undecayed galls were first detected by the senior writer in some fresh unstained thin sections which had been prepared by Dr. Townsend. Whether these were actually the bacteria we have since isolated is uncertain. These bacteria occurred sparingly in small clumps but were so unmistakable, when once actually seen, that it was agreed forthwith to make the disease a subject of further study. With this end in view Miss Alice C. Haskins, who had been trained in the senior writer's laboratory and who was then an assistant in Dr. Townsend's laboratory, was directed to make agar-poured plates from the interior of suitable galls; and, with the bacteria so obtained, inoculations on healthy daisy plants. This work proceeded for many months without positive results. Bacteria of several sorts were obtained frequently from the interior of the galls, sometimes in abundance, but all the inoculations were negative. Several factors contributed to this result. It was not then known that the true parasite comes up slowly on agar-poured plates (three to six days or more being required), nor that the tissues frequently contain a variety of saprophytes which develop rapidly on agar. Probably most or all of the inoculations of this period were made with saprophytic organisms, i. e., with those first appearing on the poured plates.

In studying the relation of bacteria to these galls, we found little encouragement in the microtome sections, either stained or unstained. The stain used in this connection was carbol fuchsin, with the result that with high powers granules could be seen in and around some of the cells, but these were few in number and did not seem to have the characteristic even outline of bacteria. This material was fixed in alcohol.

The cultural methods used by Miss Haskins were as follows: Galls were crushed in beef broth, from which agar-plate cultures were made. For this purpose, fresh, soft galls of small size were used, as well as galls of larger size and firmer texture. In preparing the galls for these cultures, the common technic of the laboratory was used, i. e., the surfaces were scraped off with a sterile scalpel; the galls were then washed for 30 seconds in mercuric chlorid water (1: 1,000), and then in sterile water. After this they were cut into small pieces with a sterile knife and placed in tubes containing 10 c. c. of neutral sterile peptonized beef broth, one tube being used for each gall. The galls were then crushed as much as possible with a sterile glass rod.

At intervals of two to four weeks during the greater part of two years agar plates were poured from preparations made in this manner. These plate cultures, which altogether amounted to several hundred, were kept at temperatures varying from 20° to 30° C. Both white and yellow colonies of different shapes and tints appeared from time to time, and some of the plates seemed to contain pure cultures, but none of the organisms were constant in all the plates from all the galls. Slant agar cultures were made, however, from a selection of such colonies as appeared, and from these subcultures inoculations were made by means of needle pricks into both old and young stems and leaves of healthy daisy plants. An occasional gall was found at or near the point of inoculation, but they were so few and so uncertain as to their formation that the inoculations were considered as having no significance.

The possibility of these growths being due to bacteria was therefore temporarily abandoned (Dr. Townsend) and various attempts were made to produce them by mechanical injuries practiced upon both young and old plants. Notches varying in number and extent were cut with a sharp knife into the sides of main stem and branches. The main stem was cut off at different distances from the ground. In some instances the entire top was removed, and in other instances only the top of the main stem was cut off. Other injuries of this nature graded between these two extremes. Branches were also cut off at different distances from the main stem, and some were simply clipped without cutting off. Parts of leaves were cut off. The main stems were injured near the base by jabbing with the point of a knife. Some stems and branches were broken off, while others were simply broken and left hanging by a portion of the tissue. In addition to the injuries mentioned, combinations of these were made upon healthy plants in various degrees of severity until we had 20 series of simple and compound injuries. These were all started in the pathological greenhouse upon plants produced from cuttings from healthy marguerites. Abnormal growths appeared on some of the injured plants, but they were not produced with any degree of regularity or certainty, and the growths rarely occurred exactly at the point of injury. Furthermore, the abnormal formation when occurring at the point of injury had more the appearance of callous growths than of the original daisy galls.

About this time (May, 1906) one of us observed, in studying microtome sections stained with anilin compounds having a strong affinity for bacteria, that while no distinct bacteria could be made out, nevertheless that part of the section lying deepest, i. e., bordering on the sound tissues, took the stain much heavier than the rest of the gall,

as though the living bacteria might be lodged most abundantly in this portion. It was suggested, therefore, that for the next series of plates deeper tissues should be used. Six sound daisy galls varying in size from 2 to 20 mm. in diameter were therefore selected by Miss Haskins and carefully washed in distilled water with a firm brush, rinsed in twice distilled water, put into mercuric chlorid water (1:1,000) for 45 seconds, rinsed in sterile water and each knot then placed in a test tube containing 10 c. c. of sterile bouillon. In cutting these galls, a small portion of the stem at the point of attachment of the gall was also removed with the gall. After placing these galls in bouillon they were cut and mashed as much as possible with a sterile knife and a sterile glass rod. Some of the more woody portions it was impossible to crush thoroughly. However, from these six bouillons, each inoculated from a separate gall, 19 agar-plate cultures were made, three from each tube except No. 1. In 48 hours all the bouillon tubes were clouded and a yellow organism developed during the same period in four of the six groups of plates; that is, four of the knots had produced agar-plate colonies in 48 hours, the plates being kept at room temperatures of from 20° to 25° C. On the fifth day after the plates were poured a few small, round, white colonies appeared in each plate in five of the six series. Slant agar and potato cylinder cultures were made from both the yellow and white colonies, also cultures in litmus milk. The indications were that three kinds of yellow colonies had formed, and that all the white ones were alike.

On June 1 inoculations were made from each of the 4 organisms into the stems of young healthy daisy plants growing in the pathological greenhouse. The inoculations were made at the top, middle, and base of the stem in each case. For this purpose young slant agar subcultures were used. The portion of the stem to be inoculated was washed with corrosive sublimate water (1:1,000) and then with sterile water. The growing organism was smeared upon the stem with a sterile platinum needle and pricked into the tissues by means of a sterile steel sewing needle. Control pricks were made with a sterile needle on other daisy plants for comparison.

On June 8 another set of healthy plants, older than the former set, was inoculated with fresh cultures of the four organisms in the manner described above.

On June 18, in the first series (those of June 1) a distinct elevation (knotty growth) was visible at each point where an inoculation had been made with the white organism, but no change had taken place in any of the plants inoculated with the yellow organisms nor in any of the control plants.

In the second series on June 23, that is, 15 days after inoculation, slight elevations were visible at all points where the white organism

was used, but no elevations were discernible at points where the yellow organisms were used nor on the control plants.

Knots of considerable size subsequently developed in some of the pricked spots, but they were not watched and the labels were lost off.

CONFIRMATORY INOCULATIONS AND CROSS INOCULATIONS.

Further inoculations by the writers of this bulletin were then made as follows with the daisy organism and with the same or similar bacteria plated from galls on other host plants, the organism from the daisy being described after a few months as *Bacterium tumefaciens* Smith and Townsend (Science, Apr. 26, 1907, pp. 671–673; and Centralb. für Bakt., 2. Abt., XX. Bd., December, 1907, pp. 89–91.)

EXPERIMENTS WITH THE DAISY ORGANISM.

DAISY ON DAISY.[a]

INOCULATIONS OF NOVEMBER 27, 1906 (BROWN).

Made 28 inoculations into marguerite daisies, using 4 different organisms plated from a daisy gall found in the greenhouse (probably produced by one of Miss Haskins's inoculations—labels lost off). Inoculated each organism into 7 different daisy plants at the tip. The cultures were 2 days old.

Result.—December 12: All 7 plants inoculated with the white organism (designated *B*) had knobby outgrowths. No protuberances were visible an plants inoculated with the other organisms.

December 18: Galls formed on all those plants inoculated with *B*. The same organism (*B*) was isolated by poured plates from one of these galls and its infectious nature proved by the following inoculations:

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[a] Wherever the word daisy is mentioned in the following pages it means hothouse varieties of *Chrysanthemum frutescens* unless otherwise stated. All of the inoculated plants were grown in hothouses unless otherwise stated. All of the inoculations recorded in this bulletin are pure-culture inoculations made with the bacteria described by us; all were made from poured-plate colonies or subcultures therefrom, usually the latter, and all the figures of galls shown in the plates are the result of such pure-culture inoculations, with the exception of a few figures introduced for comparison, viz, Plate III, upper left-hand figure (oleander from California); Plate IV, figure 1 (nematode galls on sugar beet); Plate XVI, figure 2*b* (nitrogen-fixing root tubercles on alfalfa); Plate XX (crown-gall of rose and nematode galls on Stizolobium); Plate XXIII (poplar gall from New England); Plate XXXI (hard gall on apple from Oregon and gall on blackberry from Wisconsin); Plate XXXV, figure 2 (quince gall from Algeria); Plate XXXVI, figure 2 (lettuce gall from a hothouse in Maryland). The reader who wishes to get at the positive and negative results of the inoculations quickly is advised to consult Tables II and III, beginning on page 133.

In all inoculation headings and also in the plate descriptions in such expressions as *Daisy* on Daisy, *Peach* on Peach, etc., the first word is to be understood as a convenient substitute for a phrase, e. g., "Daisy on daisy" means pure culture of a schizomycete originally isolated from a natural tumor on daisy and inoculated on daisy.

The name of the individual making the experiment is usually prefixed to it, but generally two of us were present when the results were recorded, and the authors of this bulletin are to be held jointly responsible for all statements made in it, except those relating to cancer, for which the senior writer alone is responsible.

Finally, all of our results are reported, whether favorable or unfavorable.

INOCULATIONS OF JANUARY 8, 1907 (BROWN).

Inoculated 8 plants with organism plated December 18 and designated as *B*.

Result.—In 7 days galls had started to form on each plant at the place inoculated.

INOCULATIONS OF JANUARY 18, 1907 (BROWN).

Inoculated 7 old plants on both old and young stems—24 inoculations in all. Agar culture 49 days old (white organism).

Result.—February 6: No galls had formed; cultures probably dead.

INOCULATIONS OF FEBRUARY 6, 1907 (BROWN).

Inoculated 8 more old daisy plants on young twigs only with a culture 9 days old.

Result.—February 18, 1907: Small galls had formed.

Other inoculations were made with the original cultures, as follows:

December 13, 1906.—Eight daisy plants were inoculated with cultures of the same date as those used November 27 and which had produced galls. These cultures were now 19 days old.

Result.—December 24: Galls were forming at inoculated places.

December 31: All the inoculated plants had galls. On February 23, 1907, photographs were made (Pl. I, fig. 1).

July 10, 1907: The galls had reached a large size and were quite hard (Pl. I, fig. 2).

December 21, 1906.—Eight inoculations and 4 checks.

Result.—December 31: Galls at all inoculated places; checks free; organism plated out of one of these galls and identified.

January 19, 1907.—Inoculated 7 plants with organism plated from gall on December 31.

Result.—January 30: Indications that galls will form.

February 5: Galls formed at each inoculated place.

INOCULATIONS OF FEBRUARY 18, 1907 (SMITH).

Four vigorous young plants of white-flowered Paris daisy and 6 similar plants of the yellow-flowered Paris daisy were selected. Each plant of the white variety branched at the base into two equal shoots; 7 of these shoots were inoculated and the eighth was held as a check. On the inoculated shoots also check pricks were made an inch or two above the places where the infected needle entered. All of the inoculations were made by needle pricks, using a slant glycerin-agar culture, 7 days old, which had been streaked from another slant agar culture. The organism was derived from a strain which had been passed twice through the daisy by Miss Brown with the production

of tumors (the organism designated *B*). It was probably a third or fourth subculture from the colony. Four of the inoculated white-daisy shoots received 3 needle pricks each; two received 1 prick each; one received 50 pricks. One to three check pricks were made in each case, except on one shoot, which received 50 check punctures.

The yellow-flowered daisies were each about 9 inches high and limited to a single stem. Two of them received 1 infected prick each near the top, with a check prick on each a little higher up on the same side. Two of them received 3 infected pricks each near the top, with 3 check pricks on each a little higher up. One received 30 infected-needle pricks up and down the stem. The sixth plant, held as a check, received 50 punctures up and down the stem with a sterile needle.

Result.—February 23, 1907: There was distinct evidence of infection on each of the 12 shoots at the end of 5 days, the protuberances on some being nearly a millimeter high.[a]

July: The plants were removed at the end of 1 month, 2 months, and later with well-developed tumors. Galls formed only where inoculated. The 122 sterile (check) punctures healed normally. Every infected prick resulted in a larger or smaller tumor.

November 25: During the summer some of the plants developed many secondary infections (metastases).

Inoculations of December 19, 1907 (Brown).

Seven young daisy plants were inoculated by needle pricks on the stem with agar streak cultures 2 days old. The Queen Alexandra and a large yellow variety were used for these inoculations.

Result.—January 28, 1908: No galls were formed. The plants were not in a growing condition, although they were young cuttings.

Inoculations of March 12, 1908 (Smith).

Five plants of the Paris daisy were inoculated with *Bacterium tumefaciens* from daisy on agar streak cultures 48 hours old. These plants were inoculated as controls on the inoculations from the same cultures into olive and oleander (pp. 31 and 34).

Result.—May 21, 1908: All developed tumors promptly. Only 4 of the 5 plants now remain. They have good-sized tumors.

Inoculations of February 11, 1908 (Brown).

The crowns of 6 daisy plants were cleaned and inoculated by needle pricks with a 2-day-old culture. The soil was then replaced over the inoculated places. Three other plants were punctured on the crown with a sterile needle for checks.

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[a] On older and slower-growing material inoculated by Miss Brown 12 days before the growth of the tumors was slower and they were still incipient.

Result.—March 30, 1908: Galls had formed on all the inoculated plants. The checks did not contract the disease.

DAISY ON FIELD DAISY.

Inoculations of April 15, 1907 (Brown).

Wild oxeye daisy plants (*Chrysanthemum leucanthemum* var. *pinnatifidum*) transferred from a field near Washington and grown in pots in the greenhouse, were inoculated on the young stems and also on the leaves. Four plants were inoculated in 4 or 5 places on each, and check pricks were made on one plant.

Result.—April 22, 1907: Galls had formed on all of the inoculated stems at the places pricked. None appeared on the inoculated leaves.

May 11, 1907: The numerous galls did not grow to the size of those on the cultivated daisy, the largest ones being only half an inch in diameter. The check plant did not contract the disease.

DAISY ON JAPANESE CHRYSANTHEMUM.

Inoculations of May 6, 1907 (Brown).

Three hothouse chrysanthemum plants were inoculated by needle pricks on the stems and leaves with agar streak cultures 2 days old. Check punctures were made on two other plants.

Result.—July 19, 1907: Galls had formed at all points of inoculation on the stems; none appeared on the leaves. The checks bore no galls.

DAISY ON CHRYSANTHEMUM CORONARIUM AND ON SHASTA DAISY (BURBANK HYBRID).

Inoculations of July 23, 1907 (Brown).

Six plants of *Chrysanthemum coronarium*, and 6 of Shasta daisy were inoculated by needle pricks on the lower parts of the stems with agar streak cultures 5 days old. Checks on both plants were held.

Result.—August 27, 1907: Galls had formed on all the inoculated plants at the places punctured. Those on the Shasta daisy were quite large. The checks remained free.

September 30, 1907: The inoculated *Chrysanthemum coronarium* are all dead, apparently as a result of the inoculation.

DAISY ON THE CORN MARIGOLD.

Inoculations of August 1, 1907 (Brown).

Six plants of the corn marigold (*Chrysanthemum segetum*) were inoculated on the stems by needle pricks with agar streak cultures 2 days old; two checks were held.

Result.—August 27, 1907: All the inoculated plants had galls. The checks were free.

September 30, 1907: All the inoculated plants were dead. The checks were alive.

The disease sometimes occurs naturally on this species. One such plant has been observed bearing 6 galls.

DAISY ON PYRETHRUM.

Inoculations of September 26, 1907 (Brown).

Seven full-grown plants of pyrethrum (*Chrysanthemum coccineum*) were inoculated by needle pricks on the stems with agar streak cultures 2 days old. The stems were woody and had almost ceased growing.

Result.—October 21, 1907: Small knots had formed on all of the inoculated stems. The 3 check plants were free from knots.

DAISY ON ENGLISH DAISY.

Inoculations of August 1, 1907 (Brown).

Eight seedling plants of the English daisy (*Bellis perennis*) were inoculated by needle pricks with agar streak cultures 2 days old. The stems were inoculated just below the surface of the ground.

Result.—August 27, 1907: Five of the inoculated plants bore knots; the two checks had no knots.

DAISY ON SALSIFY.

Inoculations of March 2, 1907 (Townsend).

Twelve plants of *Tragopogon porrifolius* were inoculated near the crown, using agar streak cultures of the daisy-gall organism made February 27. Six controls were made at the same time on other plants in the same relative positions.

Result.—No galls formed on either the inoculated or the control plants. In all probability these were the cultures used successfully on carnation of same date.

Inoculations of February 27, 1908 (Townsend).

Dr. Townsend made a second set of inoculations on salsify with positive results (Pl. XXII, fig. *A*), but the notes concerning this series have been misplaced. We do not know how many plants were inoculated. The one shown in the illustration was removed and put into alcohol May 8, 1908.

DAISY ON TOMATO.[a]

INOCULATIONS OF FEBRUARY 18, 1907 (SMITH).

About a half dozen needle pricks were made on each of 3 soft terminal shoots of as many tomato plants which were about 18 inches high. The organism used was an agar streak culture 7 days old (used also for the daisy inoculations of Feb. 18).

Result.—Nothing was immediately visible, but after some weeks slow-growing hard tumors developed on each one of these plants in the inoculated part.

DAISY ON POTATO.

INOCULATIONS OF MARCH 2, 1907 (SMITH).

The stems of 6 potato plants (*Solanum tuberosum*) were inoculated by needle pricks with agar streak cultures 3 days old. The plants were in pots in the greenhouse.

Result.—March 27, 1907: Galls developed at all the points of inoculation and are at this date 1 cm. in diameter. One was cut off and plates were poured from it. Four days later the characteristic colonies appeared, and daisy plants were inoculated with subcultures from these colonies. In 15 days small galls had formed on the daisies.

April 22: Stems cut off and photographed (Pl. II, fig. 5*a*, *b*).

INOCULATIONS OF MARCH 21, 1907 (TOWNSEND).

Eighteen plants of *Solanum tuberosum* were inoculated on the stems. Fourteen of these were inoculated near the tip and four near the base. A number of control punctures were made on other plants at the same time.

Result.—Galls were formed in 2 to 3 weeks at all of the inoculated points. No galls formed on the control plants.

DAISY ON TOBACCO.

INOCULATIONS OF FEBRUARY 18, 1907 (SMITH).

About a half dozen needle pricks were made on each of 3 terminal soft shoots on as many tobacco plants, which were about 3 feet high. The material for inoculation was an agar streak culture, 7 days old (used also on daisies of Feb. 18).

Result.—Nothing was immediately visible, but after some weeks slow-growing hard tumors developed on each one of these plants in the inoculated part and not elsewhere. (For microscopic appearance of a section through one of these tumors see Pl. XXIX.)

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[a] Dr. G. P. Clinton, of Connecticut, has reported the finding of crown-gall on bittersweet (*Solanum dulcamara*).

INOCULATIONS OF MARCH 20, 1907 (BROWN).

Six tobacco plants were inoculated on the stems and leaves by needle pricks with agar streak cultures 2 days old. One plant was held as a check.

Result.—April 12, 1907: Knots a half inch in diameter had formed on all of the inoculated plants on leaves as well as stems. The check remained free.

INOCULATIONS OF SEPTEMBER 19, 1907 (BROWN).

Six tobacco plants were inoculated by needle pricks on the stems with 3-day-old agar slant cultures. Four plants were held as checks.

Result.—October 25, 1907: The inoculated stems all bore knots; there were none on the checks.

DAISY ON OLEANDER.

INOCULATIONS OF MAY 6, 1907 (BROWN).

The stems of 3 oleanders were inoculated with agar streak cultures 2 days old; two checks were held. The plants were small and not in very good condition.

Result.—March 4, 1907: Small galls are visible.

July 19, 1907: Galls had formed on the inoculated plants where punctured, but they were not very large, i. e., not over half an inch in diameter; none appeared on the checks.

INOCULATIONS OF MARCH 5, 1908 (BROWN).

Four young shoots of single white oleander were inoculated by needle pricks with agar streak cultures 2 days old; two checks were held.

Result.—March 18, 1908: Galls had formed on all the places inoculated. The checks remained free from galls.

INOCULATIONS OF MARCH 7, 1908 (SMITH).

Six oleander plants were inoculated with agar slants 48 hours old.

Result.—June 1, 1908: All produced tumors.

INOCULATIONS OF MARCH 12, 1908 (SMITH).

Ten plants were inoculated with agar streak cultures 48 hours old of *Bacterium tumefaciens* from Paris daisy. The oleander plants were of three varieties: Madam Peyre, Professor Parlatore, and Single White. They were in excellent condition, 7 out of 10 of them having double shoots from the roots (young wood), 1 shoot lower and younger than the other; the other 3 bore single shoots. Where 2 shoots came from the same root or stem base, the lower, younger one

was inoculated. The inoculations were made into soft stem tissues and leaf tissues at the top of the shoot. The method was to take out a little of the agar slime on a platinum loop, rub it gently over the surface, and prick through it with a delicate steel needle, making a dozen or more light punctures. Five daisy plants were inoculated with the same culture for controls.

Result.—May 21, 1908: The shoots are now about 15 inches to 2½ feet high. Glossy swellings in the pricked areas began to appear after a few weeks and were especially pronounced on the variety known as Single White, so that at first it seemed as though the other 2 varieties would prove immune. But they soon showed distinct, small, ruptured (corky) tumors which grew slowly. The results by plants on this date are as follows:

Plant A, Madame Peyre: The top of the shoot which was inoculated has grown 5 inches beyond the punctured part. That part now bears 12 tumors, which are small but decided, there being no doubt whatever as to their nature. Their height is about 2 millimeters and their breadth about the same.

Plant B, Madame Peyre: This shoot has grown about 4 inches beyond the punctured part. The tumors have fused so that the exact number can not be stated, but there is a knobby mass where the plant was punctured. The size of the fused portion may be about 6 by 4 millimeters, and the height of it perhaps 3 millimeters, and around this are a few independent small tumors.

Plant C, Professor Parlatore: This plant has grown about 8 inches beyond the punctured part; there are 9 distinct small tumors and a couple of fused ones. Their height is about 3 millimeters, and their diameter 2 to 3 millimeters. Surface somewhat brown and roughened, which is true of all of the larger tumors.

Plant D, Single White: This shoot has grown about 3 inches beyond the punctured area. The punctured part bears about a dozen tumors, some fused; the largest is about 3 millimeters high by 3 to 4 millimeters broad, with a roughened surface. The smallest one still has the smooth, shiny, unbroken skin characteristic of all of them on the start, and characteristic also of the smallest tumors observed on the oleanders received from Fresno, Cal., this spring.[a]

Plant E, Professor Parlatore: This plant has shown less reaction than any of those hitherto examined, probably because the inoculated shoot has made less growth. The growth beyond the punctured area is only about 1½ inches, and there are only three small tumors, each about 1 millimeter high and the same in diameter. Fifteen other needle punctures appear to have given no reaction.

Plant F, Single White: This is a tall plant of a single stalk and is now in blossom. Since the punctures were made it has grown about 10 inches (beyond the pricks) and developed the blossom stalk. In all of those hitherto described the tumors have been on the stem, but 4 of the tumors on this plant are on the base of a petiole and 5 are on the stem. Three additional pricks have not developed anything. The larger of these tumors are estimated to be 3 millimeters broad and about 2 millimeters high, surface roughened. Distinct overgrowth.

Plant G, Madame Peyre: Shoot has grown about 4 inches since it was inoculated, and it bears 10 separate tumors (one fused out of about 4) and 4 punctures that have not given any distinct growth. One of the little tumors is on the base of a petiole.

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[a] The organism causing the Fresno disease is probably not identical with the one here described.—E. F. S.

The tumors have a rough, corky surface quite distinct from any wound reaction due to the needle punctures.

Plant H, Madame Peyre: Shoot has grown 3 inches since it was punctured. There are 3 little tumors on the midrib of a leaf, and 5 below this on the stem.

Plant I, Single White: This is one of the tall single-stem plants. It has grown about 10 inches beyond the point of inoculation and is budding ready to bloom. The shoot bears 9 distinct tumors, the largest of which are about 4 millimeters in diameter and about 3 millimeters high. Some are on the stem; some on the base of a petiole. Three needle punctures have failed to give any distinct growths.

Plant K, Single White: This is a tall plant, consisting of a single shoot. It has grown beyond the point of inoculation a distance of about 9 inches. The tumors are on the stem. They have mostly fused into a rough, brownish mass, but there are 2 or 3 separate ones. Their height above the surface of the green stem is perhaps 2 to 3 millimeters.

The last two plants and the one confined to a single stem (Plant F) have made much the greatest growth beyond the point of inoculation, and corresponding to this the tumors also are larger than on any of the others. This fact, taken in connection with the small, imperfect development of tumors on Plant E, which has made the least terminal growth, is very instructive and points without doubt to the conclusion that the amount of tumor growth is dependent upon the rapidity of growth of the shoot itself, i. e., the condition of nourishment of the part—a slow-growing shoot would have slow-growing tumors, but a rapid-growing shoot would develop correspondingly large ones.

These plants have now been inoculated nearly two and one-half months and have developed very good tumors for the amount of time, judging from Clayton O. Smith's statements respecting the slow growth of natural tumors on the oleander.

The Paris daisies inoculated at the same time for controls developed good-sized tumors promptly.

The final photographs (reproduced in Pl. III, figs. B, D) were made October 28, 1908.

DAISY ON OLIVE.

Inoculations of February 14, 1907 (Brown).

Two olive trees about 2 feet tall were inoculated on all the young shoots and a few old stems, with agar streak cultures 7 days old, the third subculture from the poured-plate colonies.

Result.—April 4, 1907: No knots.

Inoculations of March 11, 1907 (Smith and Townsend).

A young growing shoot of olive was inoculated with a virulent agar culture of the daisy organism (used also on this date for successful inoculations of the peach, p. 38).

Result.—Negative.

INOCULATIONS OF MARCH 12, 1908 (SMITH).

Five olive plants were inoculated with the daisy organism from agar streak cultures 48 hours old in soft wood near the growing tip of each shoot. They were held as checks on the second set of oleander inoculations (a set which produced tumors). Five daisy plants were inoculated from the same cultures for controls.

Result.—May 21, 1908: The results by plants on this date are as follows:

Plant L: Terminal shoot has grown only 2 inches beyond the 15 needle pricks; no tumors.

Plant M: Terminal shoot has grown vigorously a distance of about a foot beyond the pricked portion; about 30 needle pricks; no tumors.

Plant N: Terminal shoot has 20 needle punctures, no tumors; slightly raised rough corky places where the pricks have healed. Shoot has grown vigorously a foot beyond the needle pricks.

Plant O: Terminal shoot has about a dozen punctures, slight corky projections where needle entered. These raised portions are perhaps one-third millimeter in diameter and the healed corky portion of the wound itself possibly 1 mm. in diameter. All are alike on this and on the other plants. There are no tumors. The terminal shoot has grown over a foot beyond the point of inoculation.

Plant P: Basal shoot and terminal shoot inoculated; about 12 needle punctures. Shoots have grown about 6 inches beyond the pricks; no tumors.

November 16, 1908: No tumors.

The daisy plants inoculated at the same time all produced good-sized tumors promptly.

DAISY ON VEGETABLES (BEET, RADISH, CARROT, ETC.).

INOCULATIONS OF APRIL 26, 1907 (SMITH AND BROWN).

The vegetables were purchased at the market and taken to the laboratory, where they were washed thoroughly and inoculated. Thirty punctures, in groups of 5, were made on both checks and inoculated plants. Agar streak cultures 2 days old were used for the inoculations. The varieties and numbers of plants used were: Radish, long and round mixed, 10; turnips, 5; rutabagas, 2; parsnips, 6; carrots, 4; red beets, 3. Two to four checks were held of each variety, except the rutabagas.

Result.—June 28, 1907: A radish plant with a large irregular tumor on one side of the root was brought in on this date and photographed.

July 19, 1907: The rutabagas, parsnips, and round radishes were dead—i. e., they did not grow. Galls had formed on the inoculated beets, long radishes, and carrots. On the radishes they were 1 inch to 1½ inches in diameter. Small galls one-fourth inch across were on all red beets except one, which was about 1½ inches across. Small galls not larger than a half inch were on the carrots. The checks were free.

July 24, 1907: Additional photographs were made (Pl. IV, fig. 2).

DAISY ON EUROPEAN GRAPES.

INOCULATIONS OF APRIL 3, 1907 (SMITH AND BROWN).

Three small, slow-growing shoots of as many vines were inoculated by needle pricks from a 48-hour agar culture.

Result.—June 27, 1907: One only of the three vines developed a tumor—a small growth about half an inch long, one-fourth inch broad, and perhaps one-eighth inch high (Pl. V, fig. 1). It was on Muscat Hamburg. The failure of the other two (Golden Hamburg and Champion Hamburg) is attributed to poor condition, the plants having made scarcely any growth.

INOCULATIONS OF APRIL 11, 1907 (BROWN).

A Golden Hamburg and Champion Hamburg were each inoculated in the laboratory with agar streak cultures 5 days old. The plants were small and covered with scales, which were removed before inoculating. The plants were then set out in the greenhouse.

Result.—May 1, 1907: The Golden Hamburg was dead; the other had made no growth and no gall had formed.

INOCULATIONS OF MAY 9, 1907 (BROWN).

A dozen Black Hamburg vines were taken from the pots, washed carefully, and 9 of them inoculated with agar streak cultures 4 days old. They were well-rooted cuttings. The inoculated plants were treated all in the same way, each receiving 20 to 25 punctures on the green shoot, underground stem, and young root. The checks were punctured in the same manner with a sterile needle.

Result.—July 20, 1907: Small galls had formed on each inoculated plant, but only on the green shoots. The galls produced were not like the regular grape galls. The checks did not develop galls.

INOCULATIONS OF MAY 14, 1907 (BROWN).

Young, well-rooted cuttings 3 to 4 inches tall were taken from the pots, washed carefully, and 9 plants inoculated on both root and shoot, 20 to 25 punctures being made on each. The cultures used were 3-day-old agar streaks. Three checks were held. The vines were afterwards repotted.

Result.—July 20, 1907: Knots had formed on all of the shoots inoculated. The checks were free from knots.

INOCULATIONS OF JUNE 9, 1907 (TOWNSEND).

Vine No. 490, Black Hamburg, was inoculated with a 4-day-old agar culture.

Result.—June 27, 1907: The plant died. It was pulled up and no tumor found on the stem.

INOCULATIONS OF AUGUST 9, 1907 (BROWN).

Eight plants were inoculated by needle pricks on the youngest parts of the stems with agar streak cultures 3 days old. Three checks were made. The varieties used were Prince Albert, Black Prince, and White Tokay. The vines were 2 feet tall and had made rapid growth.

Result.—August 27, 1907: Small knots had formed on all but 2 plants. Of the latter, 1 was a White Tokay and the other was a Black Prince. No knots formed on the checks.

INOCULATIONS OF MARCH 7, 1908 (SMITH).

Three plants of *Vitis vinifera*, 1 each of varieties Prince Albert, Barnes Muscat, and Black Prince, were inoculated by needle pricks in two places on each with 48-hour agar slants of the daisy organism.

Result.—One of the 3 developed a small tumor. The plants made very little growth.

DAISY ON AMERICAN GRAPES.

INOCULATIONS OF APRIL 3, 1907 (SMITH AND BROWN).

Four varieties of grape were inoculated with 2-day-old agar streak cultures. Thirty punctures were made on each in groups of 10—on the root, on the underground stem, and at the base of young shoots near the top of the stem. Two plants of each variety were inoculated and 1 of each held as a check, the check receiving the same number of pricks from a sterile needle. The varieties were as follows: Moore Early, Delaware, Concord, Martha.

Result.—May 9, 1907: A small gall was found on the underground stem of a Martha grapevine. No galls found on the others. All were dormant when inoculated.

DAISY ON IMPATIENS.

INOCULATIONS OF MAY 26, 1908 (SMITH).

One young growing plant of *Impatiens sultani* was inoculated from an agar streak culture 4 days old. The plant was punctured on the soft stem in several places. A daisy plant inoculated from the same culture was held for control.

Result.—No galls resulted. The plant was under observation for several months. No record respecting the control.

INOCULATIONS OF APRIL 4, 1910 (BROWN).

Five shoots of a red-flowered plant and 7 shoots of a coarser growing white-flowered sort were inoculated by needle pricks, using one of the actively pathogenic recent isolations from daisy.

Result.—June 24, 1910: All negative.

DAISY ON CLOVERS AND ALFALFA.

INOCULATIONS OF MARCH 12, 1908 (BROWN).

The roots of 2 white clovers (*Trifolium repens*), 2 red clovers (*T. pratense*), and 2 alfalfa plants (*Medicago sativa*) were inoculated by needle pricks with agar streak cultures 3 days old. Two checks were held on the clovers. The inoculated roots were marked by strings tied around each one below the point of inoculation.

Result.—March 27, 1908: Galls had formed at the inoculated points, but could not be distinguished at this date from the regular tubercles on clover.

May 14, 1908: The plants were dug up and the marked roots examined. Small galls one-fourth to half an inch across and quite distinct in appearance from the nitrogen tubercles were now present on the inoculated roots where the needle pricks were made. One gall had many projecting hairlike roots, making it resemble the apple or peach gall of the hairy-root type.[a]

INOCULATIONS OF MAY 26, 1908 (SMITH).

Five plants of scarlet clover (*Trifolium incarnatum*) were inoculated on the fleshy roots with the daisy organism from agar streaks 4 days old. These plants were dwarfed in 3-inch pots, but stood on earth and had rooted into it beneath the pots. The crowns were uncovered, then inoculated, and repotted in 6-inch pots. The plants had each a half dozen or more shoots, and were about 8 or 10 inches high. Those which had not yet blossomed were selected for this experiment. A daisy plant inoculated from the same culture was held for control.

Result.—Negative. Plants growing slowly and probably too old. Their roots were also injured in repotting.

According to Mr. Karl F. Kellerman (verbal communication), a gall of a similar character to that obtained by us occurs naturally on clover in some parts of the United States, and had been a source of confusion to him.

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[a] Viala has figured a galled vine shoot (broussins) bearing also aerial roots.

DAISY ON PEACH.[a]

INOCULATIONS OF MARCH 11, 1907 (SMITH AND TOWNSEND).

Received 27 one-year-old peach trees from Arlington Experimental Farm; washed the roots very thoroughly in running tap water for half an hour, with hand rubbing, then rinsed thoroughly twice in distilled water. All were free from crown-gall and otherwise sound.

Held 9 as check plants, making 20 needle pricks in the crown of each one, i. e., in the bleached part of the stem just below the earth surface. Divided the other 18 into two groups. One group was inoculated with a quite viscid 5-day-old culture on ordinary slant agar. The other 9 were inoculated with a 6-day-old culture on slant glycerin agar. Each one of the inoculated plants received 10 pricks (5 on one side and 5 on the other), mostly in the white tissues of the crown of the plant, but a few lower down in the taproot. They were then taken to the hothouse and planted in good earth in 10-inch pots. Young daisy plants were pricked for control (1 from each culture).

One culture was also pricked into a young shoot of olive with negative results, as already recorded (p. 33).

Result.—March 29, 1907: Nos. 33 and 37 were dug and photographed (at end of 18 days).

April 3, 1907: The roots of all the trees were examined, and 15 out of the 18 were found with tumors where inoculated, the largest being about one-fourth inch across. Five of the roots had 2 tumors each. All inoculated with the younger culture, with one exception (No. 32, a dying tree), developed tumors. Of the other 9, 7 showed tumors. What appeared to be incipient tumors were also found on the roots of the 2 trees counted as negative, so that probably all of the inoculated plants, except the dying one, would in the end have shown well developed tumors. On these 18 trees there were no tumors when inoculated, nor afterwards, except where the infected needle entered.

April 5, 1907: The 9 check plants punctured on March 11 as controls for the inoculations were dug and examined. No tumors were found on the roots of any of them. They were repotted and returned to the house. The daisy controls had tumors.

January 29, 1908: After their examination on April 3, the inoculated trees were repotted but made very little growth for a number of weeks. This setback we now know to be very injurious to the development of galls on roots. To-day the trees were dug to be thrown away, and the following conditions were observed:

On No. 36 a large gall just underground, the same being about 2½ inches in diameter and nearly encircling the root (Pl. VI, fig. 1).

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[a] See also daisy under "Peach on Peach" (p. 66), check inoculations of December 5, 1907.

Most of the others have smaller galls, some of which have rotted away except at the base. These galls are about 1 inch in diameter. No. 22 had gall about one-fourth inch in diameter. No. 25, gall not larger than last spring. No. 26, gall about one-half inch in diameter (these two are of the lot marked negative in April). No. 30 has two galls about one-fourth inch in diameter. No. 34, scars of small galls. No. 38 has no gall now visible—i. e., it has recovered.

INOCULATIONS OF APRIL 6, 1907 (SMITH AND BROWN).

Sixty-nine peach trees were brought to the laboratory from the Arlington Experimental Farm and washed carefully. All were free from natural galls and were from a soil supposed to be uninfected. They were labeled Nos. 90 to 158, inclusive. The first 21 (Nos. 90 to 110) were held as checks, being punctured on the roots with 20 needle punctures each, in groups of 5. The remaining 48 (Nos. 111 to 158) were inoculated with agar streak cultures. Daisy plants were inoculated with the same cultures for control.

The roots of 24 trees were inoculated by Dr. Smith by means of a needle, giving 15 pricks in 3 groups of 5 each (2 or 3 trees had more). For this purpose he made use of ordinary brown moderately viscid peptone beef agar cultures, 5 days old, and of white glycerin agar cultures, 5 days old, also moderately viscid.

Miss Brown inoculated 24 trees, giving 15 pricks in 3 groups of 5 each on the roots.

Dr. Smith used 6 slant agar tubes (3 of each sort) as above. Miss Brown used 6 slant agar tubes of the two sorts of agar (3 tubes of each), each 48 hours old and not yet viscid. Work done in laboratory and very thoroughly. Plants set in hothouse.

Result.—July 12, 1907: Dug to-day, brought in, washed, and examined all of the peach trees which were inoculated on April 6. They fall into three groups, as follows:

(1) Plants showing no tumors.

(2) Plants on the roots of which small tumors have developed.

(3) Plants on the roots of which larger tumors resembling the ordinary crown-gall of the peach have developed. None of the tumors are over one-half to three-fourths inch in diameter, i. e., they are not full grown.

Of the 13 uninfected plants (showing no tumors whatever) 5 were inoculated from the older cultures, 8 from the younger.

On the 17 plants showing small tumors the galls vary in size from that of a small shot to a small pea. Six of these were inoculated from the older cultures, and 11 from the younger. The tumors are all on the main root, corresponding, so far as can be determined, to

the position of the needle pricks. They vary in number from 1 to 3 on each plant.

Of the 18 plants showing larger tumors 13 were inoculated from the older cultures and 5 from the younger. All the tumors are on the main root, with the exception of one, which is on a small side root, and appears to be a secondary infection. The others seem to be primary infections, but not every group of needle punctures resulted in a gall. The tumors on these plants vary in number from 1 to 4. On plant 137 (which received 30 pricks in 6 groups) only one tumor resulted.

General remarks.—Of these plants 73 per cent show tumors. The plants were neglected on the start, receiving too little water, and for this reason made a very slow growth for several weeks after they were planted in pots in the hothouse. They have also frequently since that date received too little water.

Photographs were made of the best of this material (Pl. XI, fig. 2).

Respecting the group which shows no tumors, it may be stated that there is some evidence to show that some of those marked negative may have developed little tumors which afterwards perished. The bulk of the tumors are still sound, but a dozen or more have decayed more or less, and a few pretty completely; and if the same thing had happened to much smaller tumors on the first group, then there would be now no evidence of infection, although there might have been evidence two months ago.

Considering the slow growth of the trees the results are fairly satisfactory, especially since all the 21 check trees (420 punctures) have remained entirely free from tumors, although the trees made more growth than the inoculated ones which developed the galls. The check trees have been in the same hothouse, but removed about 30 feet from the inoculated ones. The soil was the same.

DAISY ON ALMOND.

Inoculations of March 7, 1908 (Smith).

Eight seedling hard-shell almonds were inoculated at the crown with 48-hour-old agar slants of the daisy organism.

Result.—March 18, 1908: One of the inoculated almonds was dug, and a small, well-developed tumor found at the entrance of the needle.

March 28, 1908: Two more plants dug. Nothing definite found.

March 31, 1908: The remainder of the plants were dug; 3 were found with small tumors; 2 without. The plants have stood from the time they were germinated in clean sand, and only when they were inoculated was an inch of gardeners' earth put on top of the

sand. They have, therefore, been under conditions such as would not produce a rapid growth. The inoculated tissues were rather woody, and this probably explains the small number of infections (50 per cent).

DAISY ON RASPBERRY.

INOCULATIONS OF APRIL 12, 1907 (SMITH).

Thirty-two raspberry plants, 16 of the Cuthbert variety and 16 of the King variety, were inoculated with agar streak cultures 6 days old, 15 to 20 punctures being made on each plant. Eight plants of each variety were held as checks. The inoculations were made in the laboratory and the plants immediately afterwards set out in pots in the greenhouse.

Result.—June 27, 1907: These plants were from a nursery in Virginia. They were small and not very satisfactory to work with, and they grew badly when potted, partly because they were of inferior stock and partly because of insufficient water at times. For these reasons a good many of them died on the start. The conditions, therefore, were unfavorable to the success of the inoculations.

Thirteen plants of the King variety and 6 of the Cuthbert produced no tumors. Ten plants were infected (2 King, 8 Cuthbert) with 32 tumors, many at the point of inoculation. Three plants were missing.

The tumors on all these plants are white and growing, except one or two which are decaying or dead. The largest at point of inoculation were 10 by 10 by 10 mm., 15 by 15 by 10 mm., 18 by 14 by 14 mm., and 20 by 15 mm.

July 2, 1907: The checks were brought in and examined. They had been in another hothouse adjoining the one where the inoculated plants were kept. They proved to be badly infected, so that no conclusions could be drawn from the experiment. There was no possible danger of infection from the cultures used, because they were not opened, nor any inoculations made, until after the check plants were punctured with a sterile needle and set out in the other house. The checks have grown more than the inoculated plants and the infection was probably brought along with them from the nursery, because one or two knots were found on the roots of these plants when they were purchased.

The details on checks are as follows: Eight Kings, 4 diseased with 5 tumors; 7 Cuthberts, 5 diseased—on No. 298 whole root occupied, about a dozen tumors as big as peas and others like filberts in clusters. On the others, 13 tumors. One of the two plants free from galls was dead.

DAISY ON BLACKBERRY.

Inoculations of April 11 and 12, 1907 (Smith and Brown).

Thirty-four blackberry plants, 17 of Ena variety and 17 of the Rathbon variety, were inoculated with agar streak cultures of the daisy organism, the former with 5-day-old cultures, the latter with 6-day-old cultures, each plant receiving 15 to 20 punctures. Seven checks were held of each variety. The surface of the streak cultures used for these inoculations was smooth and wet-shining; they had not spread very widely over the surface of the streak—widest, however, near the fluid in the V. The fluid itself was thinly clouded, except at the top, which had stringy white masses of bacteria. The inoculations were made in the laboratory and the plants immediately afterwards set out in pots in the greenhouse. The plants were obtained from Virginia.

Result.—June 27, 1907: Three of the Rathbons dug; no tumors. One was dead when dug.

July 3, 1907: The remainder of the inoculated blackberry plants were dug and the roots examined. No tumors were found. Twenty of the plants were dead. The others had made a moderate growth.

DAISY ON APPLE.

Inoculations of April 13, 1907 (Smith).

Three varieties of apple (2 trees of each) were inoculated with the daisy organism, each tree receiving 30 pricks, in groups of 5. The varieties were as follows: Baldwin, Early Harvest, Ben Davis (No. 386 had hairy knots on its roots, which were pruned off).

Six trees were held as checks, each receiving 30 pricks in groups of 5, as follows: 2 Baldwin; 2 Early Harvest, 2 Ben Davis.

Result.—June 28, 1907: Early Harvest, No. 382—A few slight calluses on the cut surface of the root; no indication of galls in the pricked areas or elsewhere. Baldwin, No. 358—Similar to the preceding; nothing suggestive of tumors. Ben Davis, No. 386—Much more decided evidences of tumors; nearly every root which was pruned back has an abnormal amount of callus, resembling a gall, on its cut surface, and there are also some little galls on one root; no evidence of any tumors where the needle entered—in fact, it is difficult to find where the needle did enter.

July 13, 1907: The remainder of the apple trees inoculated April 13, 1907, were dug, the roots washed and examined for galls. Condition of roots as follows: Early Harvest, No. 381—Badly overgrown calluses. Ben Davis, No. 387—Very badly overgrown calluses, seven of them. Baldwin, No. 357—Overgrown calluses.

Remarks.—No positive conclusions can be drawn from this experiment, since at least some of the trees came from infected soil, as shown by the hairy root.

INOCULATIONS OF MAY 26, 1908 (SMITH).

Five vigorous shoots of Wealthy apple (in the hothouse) were inoculated near the tip with the daisy organism from an agar streak 4 days old. As a check on these a daisy plant was inoculated in two places from the same tube used to inoculate the apple shoots.

Result.—June 1, 1908: The shoots are vigorous—about 3 feet long. The daisy is developing tumors in both the places inoculated. Apple tumors, therefore, should be obtained later. Nothing definite now.

September, 1909: No tumors appeared on the apples.

INOCULATIONS OF JULY 20, 1910 (SMITH AND BROWN).

Trees 1 and 2 years old were inoculated on shoots and on crowns by needle pricks from young agar cultures of a recent isolation.

Result.—October 22, 1910: Negative above ground and uncertain below. Numerous galls were present on the crown and roots of a number of the trees, but *Schizoneura lanigera* was present.

DAISY ON ROSE.

INOCULATIONS OF MARCH 27, 1907 (BROWN).

Very young rose shoots were inoculated by needle pricks with slant agar cultures 2 days old. The shoots of 3 plants were punctured with a sterile needle for checks.

Result.—April 19, 1907: Small knobbed protuberances were formed at each inoculated place; the checks were free from knobs.

INOCULATIONS OF APRIL 3, 1907 (SMITH AND BROWN).

Eighteen rosebushes were inoculated with agar streak cultures 2 days old. The plants, including the 6 healthy checks, were washed thoroughly in running water. The varieties were Bridesmaid and Bride. Nine plants of each variety were inoculated and 3 checks held of each variety. Each plant received 10 to 20 punctures. Some were inoculated at the base of the shoot on the main stem, some on the stem below ground, and some at the base of young shoots. All were growing slowly. The plants were in pots in the greenhouse. Daisy plants were inoculated for control.

Result.—May 9, 1907: No galls on the rosebushes. The daisy controls developed galls.

DAISY ON VARIOUS ORCHARD TREES.

Inoculations of April 13, 1907 (Smith and Brown).

The following varieties were used, each receiving 30 pricks in groups of 5: Windsor pear, Sheldon pear, Bartlett pear, Worden Seckle pear, Wickson plum, Abundance plum, Montmorency cherry, Black Tartarian cherry, Harris apricot, J. L. Budd apricot, soft-shell almond, and American chestnut.

The trees were for the most part overgrown and in bad condition when received from the nursery, but bore no root knots or crown-galls. They were brought to the laboratory, the roots scrubbed, and then inoculated with young agar streak cultures; 2 of each sort were inoculated and 2 were held as checks.

Result.—June 10, 1907: The almond trees died without leafing out. When pulled up and examined to-day no tumors were found on the roots. Most of the chestnut trees were also dead and dying. They leafed out a little bit, but not to any great extent. No tumors were found on the roots. The two trees of Black Tartarian cherry were also pulled up and examined. There were no tumors on the roots. They had leafed out a very little and then died.

July 13, 1907: The remainder of the trees were dug and examined for galls. Condition of the roots as follows:

Worden Seckle pear—No. 363, small root tumor and several badly overgrown calluses, which are like tumors; No. 364, overgrown calluses.

Sheldon pear—No. 397, small tumors scattered along root; No. 398, badly overgrown calluses.

Bartlett pear—No. 379, small tumors along root and tumefied calluses badly overgrown; No. 380 (which is smaller than the others) bears on its roots 3 well-developed, typical root tumors. The largest one is connected with the root by a small pedicel and is over an inch in diameter. The Bartlett pear seems to be quite susceptible.

No tumors resulted from the inoculations on any other of the trees used in this experiment, but the evidence is of little negative value owing to the character of the trees at the time of inoculation. We did not at this time understand the necessity of inoculating into growing tissues.

DAISY ON CABBAGE.

Inoculations of March 29, 1907 (Brown).

Young cabbage plants were inoculated on the leaf blades with 4-day-old agar cultures isolated from the daisy.

Result.—April 15, 1907: Knobbed growths developed at all the places of inoculation.

INOCULATIONS OF APRIL 18, 1907 (SMITH AND BROWN).

Two cabbage plants were inoculated on the midribs of a dozen outer leaves; checks were held on another plant.

Result.—April 26, 1907: Each one of the inoculated midribs had split, and on these splits were knobbed outgrowths. The checks remained free from splits and knobs.

June 28, 1907: A cabbage stalk brought from the hothouse showed numerous tumors growing out of the leaf scars, the lower (inoculated) leaves having fallen. These appear to be secondary infections. Young sprouts are growing out of the tumors. Several other plants (none of which were inoculated on the stems, but all on the leaves) at this time showed similar tumors growing out of the leaf scars.[a]

INOCULATIONS OF MARCH 7, 1908 (SMITH).

Three leaf scars on each of three cabbage plants were inoculated with 48-hour agar slants of the daisy organism.

Result.—Nothing definite.

DAISY ON CARNATION.

INOCULATIONS OF MARCH 2, 1907 (TOWNSEND).

Twelve inoculations were made into stems of carnation (*Dianthus caryophyllus*), using agar streak cultures of February 27. Ten of these inoculations were made near the growing tips and two about midway of the older stems. Six controls were made in the same relative positions on other plants.

Result.—Knots or galls formed in all cases at the points of inoculation in times varying from two to six weeks. No galls formed on the controls. One of the galls was photographed September 18 (Pl. VII, fig. 1).

DAISY ON SUGAR BEET.

INOCULATIONS OF APRIL 17, 1907 (BROWN).

A row of young sugar beets about 4 inches high growing in the greenhouse was used for these inoculations. The soil was turned back from the root, the root washed with sterile water and inoculated with agar streak cultures 2 days old; the punctured places were covered with moist cotton, and the soil replaced. Twelve plants were treated in this way, and three were punctured with a sterile needle for checks.

[a] The senior writer secured infections in the laboratory on the freshly cut surface of raw turnip roots kept in deep sterile Petri dishes. The bacteria were rubbed on the surface with a platinum loop after the root had been scrubbed, soaked for an hour in mercuric chloride water (1:1,000) and cut with a sterile knife (Pl. IX, fig. 2). A yellow turnip bearing galls not due to nematodes was received from Texas.

Result.—April 29: Some of the inoculated plants bear galls, and one was photographed.

May 9, 1907: Galls half an inch in diameter were found on all of the inoculated beets; the checks had no galls.

May 29, 1907: The galls had increased in size so they were now 2 to 3 inches across.

INOCULATIONS OF NOVEMBER 15, 1907 (SMITH).

Twenty-four sugar beets (Nos. 500–519 and 523–526) were inoculated with the daisy organism from slant agar cultures of November 11, 1907. The portion of the beet projecting above ground was washed carefully with a clean cotton plug wet with filtered water, after which a large amount of milky fluid from the slant agar cultures (into which about one-half c. c. filtered sterile river water had been poured) was put on the cleaned surface with a sterile platinum loop. Ten needle pricks, about one-fourth inch deep, were made through this (close together), and more of the milky fluid was added. Three daisy plants were inoculated as checks on the virulence of the cultures, one from each tube. The daisies were Nos. 520 to 522. The sugar beets were from seeds planted in July, and were growing in pots in the greenhouse. They had made a very fair growth and were in good condition. They had good foliage, and the portions inoculated were soft and not woody. The daisies were vigorous young plants about 10 inches high, with very tender stems, and were growing rapidly. They were grown from cuttings and were in excellent condition for inoculation.

Result.—December 4, 1907: Some of the sugar beets inoculated November 15 were examined carefully and tumors as large as small peas were found on each one. A number of the sugar beets show as many tumors as there were needle pricks, i. e., 5 or 6. Some days previous very young knots were cut out of these plants, to fix for chromosome sections. The knots were at that time 10 days old and about one-tenth part as large as an ordinary pea, or perhaps even smaller than that. The daisy plants inoculated as checks showed tumors.

January 29, 1908: All of the sugar beets inoculated on November 15 produced well-developed tumors, except Nos. 509 and 524, i. e., 92 per cent. The tumors on 3 of the beets were rotting.

INOCULATIONS OF NOVEMBER 18, 1907 (SMITH).

Thirty-six sugar beets (Nos. 527 to 562) were inoculated with slant agar cultures of November 15, 1907 (from slant agar cultures of November 11, 1907, used for the preceding inoculation). The beets were from the same lot as those inoculated November 15, and were

growing well and all were free from natural tumors. The soil was removed from one side of the plant, leaving about 1 inch of the root exposed. This was rubbed with clean cotton wet with filtered water and then inoculated. A large quantity of the milky fluid from the slant agar cultures to which sterile water had been added was put on the clean surface, 10 needle pricks about one-fourth inch deep made through it, and more of the bacterial fluid put on. Six rapidly growing daisies (from cuttings) were inoculated near the tip of the stem, as checks on the virulence of the cultures.

Result.—December 4, 1907: Some of the sugar beets were examined carefully, and tumors found on all examined. The tumors were as large as small peas (16 days). Quite a number showed as many tumors as there were needle pricks, i. e., 5 or 6. All of the daisy plants inoculated as checks showed tumors.

January 29, 1908: All of the 36 beets developed tumors in the pricked area. One plant attacked by nematodes (none of the others were) also developed a second bacterial tumor about half an inch in diameter near the basal part of the root. The other 35 plants were attacked only where inoculated.

Miss M. L. Shorey isolated oxidases and peroxidases (black substances) from these tumors. These were copiously inoculated into the roots of numerous sound sugar beets, but no growths appeared.

Inoculations of June 11, 1908 (Smith).

Five sugar beets were inoculated with the daisy organism after passing it through oleander (with production of tumors) and plating it out again.

Result.—August 10, 1908: Every one of the 5 plants has a tumor at the place inoculated and not elsewhere.

Inoculations of December 4, 1909 (Brown).

Eight young sugar beets were inoculated with 2-day-old agar cultures of the daisy organism just plated from a gall; the cultures used were the first subcultures made.

Result.—December 21, 1909: No galls had formed. The soil was very cold and the temperature constantly low in the greenhouse.

April 4, 1910: Galls had formed on each one of the 8 inoculated sugar beets (Pl. VIII). Some of these galls were 5 inches across.

Remarks.—Out of a total of 85 sugar beets inoculated (5 experiments) 83 contracted the disease and 82 of them only at the point of inoculation. In 4 of the 5 experiments 100 per cent of the inoculated plants contracted the disease, pure cultures being used. Numerous uninoculated beets in the same houses remained entirely free from the disease.

DAISY ON HOP.

Inoculations of April 8, 1907 (Smith and Brown).

Two varieties of hop were used: The English Cluster and the Custis Late. The plants were knocked out of the pots, brought to the laboratory, and washed carefully. All were free from tumors. They came from New York. Eight of the English Cluster and 10 of the Custis Late were inoculated by means of needle pricks (20 to 40) on each fleshy root in groups of 5, with viscid agar streak cultures 4 days old. Four checks of each variety were held, each receiving the same number of punctures as the inoculated plants. The vines were then taken back to the hothouse and set in 10-inch pots.

No checks were made into daisy plants (1) because none were convenient, and (2) because the 4 tubes used in this experiment are part of the same lot used for inoculating peaches on April 6, and checks were kept on 12 daisy plants at that time (p. 39).

Result.—May 6, 1907: Knots were on the crowns of each of the 18 inoculated plants where punctured. The 8 checks were free.

July 11, 1907: The vines have grown slowly. The results are as follows:

ENGLISH CLUSTER.

No. 163: Four tumors on the roots, each one-half inch or more in diameter.

No. 164: Two small tumors on the upper part of the root.

No. 165: Two tumors on the upper part of root, each about as large as a wax bean; one on one side of the root and the other on the opposite side, evidently corresponding to the pricked places; midway between the two a small tumor is breaking through the root.

No. 166: One small tumor about as big as a pea.

No. 167: A decaying tumor at the top of the root and a smaller sound one a few inches down.

No. 168: A tumor about 1¼ inches long on the upper part of the root; really a multiple tumor, two having fused.

No. 169: Two tumors on the base of the stem; the larger is half an inch or more in diameter and the other about half as large; none on the roots.

No. 170: Three tumors on the upper part of the roots; 1 about an inch in diameter, 1 about half that size, and 1 quite small. The smallest is on a side root about 1½ inches below the largest one.

CUSTIS LATE.

No. 177: A tumor about an inch in diameter (longest way) at the top of the root; 4 smaller tumors scattered along the roots. The lowest one is at least a foot below the crown, and the root that bears this bears two others.

No. 178: This plant has made scarcely any growth, the two crown shoots being not more than a few inches long with diminutive leaves. Two small root tumors, each about as big as sweet-pea seeds.

No. 179: Seven root tumors, the largest over a half inch in diameter, the others about as big as small peas. Some are on the main root, some on the small side roots.

No. 180: Four tumors; 3 about as large as beans and 1 small; 3 of the 4 are on the stems near the roots; the smallest is on the root.

No. 181: A tumor on the root about 6 inches from the top; not larger than a small pea.

No. 182: Two tumors on the upper part of the root, each about a half inch in diameter; also a small tumor on a side root not larger than a sweet-pea seed.

No. 183: Plant missing.

No. 184: Two small tumors on the roots underground; one is not larger than a small pea and the other a little smaller.

No. 185: Four tumors on the upper part of the roots; 1 is three-fourths inch in diameter; another, a small tumor, is on the base of a young shoot which is tumefied over a distance of 2 inches toward the base, and it is probable that the organism had to do with this tumefaction. Saved separately in alcohol for sections.

No. 186: This is a defective, slow-growing root; it has a tumor about as large as a pea on the main root 4 inches from the top.

No. 187: Two tumors on the upper part of the root where stems come out and 1 just above on another stem; each half an inch or more in diameter.

Remarks.—Eighteen plants, all diseased; jar of material saved in alcohol; photographs made; 8 check plants subsequently examined—all free. These check plants received more than 200 punctures.

Inoculations of April 10, 1907 (Brown).

Two more varieties of hops were inoculated, the Red Canada and the Humphrey. The plants were left in the pots but the soil was turned back and the crown and young shoots were washed with sterile water. Eighteen of the Red Canada variety were inoculated, 9 with agar streak cultures 4 days old, and 9 with cultures 6 days old. Seven of the Humphrey were inoculated with a 6-day-old culture. Twenty to 30 needle pricks were made in the base of young shoots and in the crown. Two checks of each variety were held, the same number of pricks being made with a sterile needle.

Result.—April 27, 1907: All the 25 inoculated plants had galls except one, which was dead. Most of the galls were on the crown; only a few occurred at the base of the shoots. The 4 checks had none.

July 25, 1907: Removed the last of the inoculated hops (Pl. IX, fig. 1). The galls were three-fourths inch to 1½ inches in diameter. The checks remained free.

Remarks.—Of the 43 inoculated hops, 42 contracted the disease, or 100 per cent, if we exclude the 1 feeble plant which died soon after inoculation. Of the 12 checks, all remained free from the disease.

DAISY ON FIG.

Inoculations of August 9, 1907 (Brown).

Eleven young trees of *Ficus carica* were inoculated by needle pricks with agar streak cultures 3 days old. The plants were grown from cuttings and were about a foot high. The youngest and softest parts of the stems were inoculated. Three checks were held.

Result.—August 27, 1907: No galls had formed. (None formed later.)

INOCULATIONS OF MARCH 7, 1908 (SMITH).

Three fig plants were inoculated in the young tender tissues of the growing part of the stem from 48-hour agar slants.

Result.—June 1, 1908: No tumors have developed.

September, 1909: No galls appeared.

INOCULATIONS OF APRIL 6, 1910 (BROWN).

Twelve young rapidly growing shoots of an edible fig were selected and the inoculations made by needle pricks from a young agar streak culture of the newest isolation from daisy.

Result.—June 25, 1910: All negative.

DAISY ON CHESTNUT.

INOCULATIONS OF MARCH 7, 1908 (SMITH).

Three Paragon chestnut plants and 1 American chestnut were inoculated with 48-hour agar slants of the daisy organism. The needle pricks were made on growing shoots.

Result.—September, 1909: No galls appeared.

INOCULATIONS OF APRIL 7, 1910 (BROWN).

The newest isolation from daisy was employed, 4 of the chestnut plants being inoculated on the crown and 6 on young shoots. All were needle-prick inoculations from young agar streaks.

Result.—June 25, 1910: All negative. The shoots grew rather slowly.

DAISY ON OAK.

INOCULATIONS OF APRIL 7, 1910 (BROWN).

Eight small seedling red oaks (species ?) were inoculated by needle pricks into terminal slow-growing green shoots.

Result.—June 24, 1910: All negative. The shoots made only an inch of growth beyond the pricks.

DAISY ON PERSIAN WALNUT.

INOCULATIONS OF APRIL 17, 1907 (SMITH AND BROWN).

The roots of 6 trees of Persian walnut (*Juglans regia*) were inoculated with agar slants 5 days old, each receiving 30 pricks in groups of 5. Four trees were held as checks. The roots of all the trees were washed thoroughly in the laboratory before inoculating. The trees were then planted in the greenhouse.

Result.—July 13, 1907: The trees were an inch or two in diameter at the base when inoculated and have made a slow growth. No tumors resulted from these inoculations.

INOCULATIONS OF MAY 26, 1908 (SMITH).

Three vigorous young shoots of *Juglans regia pendula* were inoculated near the growing end with 4-day-old agar streak cultures of the daisy bacterium.

Result.—July 15, 1908: The 3 shoots developed small tumors in the pricked area within a week or 10 days, and these have continued to grow slowly ever since. Two shoots were put into alcohol July 15.

DAISY ON WINGED HICKORY.

INOCULATIONS OF MAY 26, 1908 (SMITH).

Eight young, actively growing shoots of *Pterocarya fraxinifolia* were inoculated by needle pricks near the growing point with 4-day-old agar streak cultures of the daisy bacterium. The tree stood on the grounds of the Department of Agriculture.

Result.—July 15, 1908: The Pterocarya proved much more resistant than the Persian walnut. There was nothing on any of the shoots for a month. More recently a few small tumors have developed on 3 of the 8 shoots. The others show nothing. Two were put in alcohol.

DAISY ON GRAY POPLAR.

INOCULATIONS OF APRIL 17, 1907 (SMITH).

Five gray poplar trees (*Populus canescens*) were inoculated with agar slants 5 days old, each receiving 30 pricks in groups of 5. Four trees were held as checks. There was a natural tumor at the earth's surface on No. 401, which was cut off. One also on No. 403 was left to be photographed. The checks received 60 pricks each in groups of 5. The roots of all the trees were washed thoroughly in the laboratory before inoculating. The trees were then planted in the greenhouse.

Result.—July 13, 1907: The trees were in a poor condition when received from the nursery. No tumors resulted from these inoculations, probably because the trees made too slow a growth.

INOCULATIONS OF MAY 25 AND 26, 1908 (SMITH).

Some young sprouts (cuttings rooted some weeks previous) were inoculated May 25 with 4-day-old agar streak cultures of the daisy bacterium.

One shoot on a tree was inoculated May 26 from the same cultures used on May 25.

One daisy plant was inoculated in 4 places from each of the 4 tubes used as a check on their virulence.

Result.—June 1, 1908: Evidence of tumors on 1 shoot (Z) at the place where the punctures were made. The daisy is developing tumors.

INOCULATIONS OF JUNE 9, 1908 (SMITH).

The inoculations were made on branches of 2 potted plants of *Populus canescens* several feet from the ground with 4-day-old agar cultures of the daisy organism.

Result.—November 16, 1908: Five small tumors have resulted; the largest is about 1 centimeter in diameter. This indicates clearly that gray poplar is also susceptible to the disease.

December 24, 1908: Branches were photographed (Pl. V, fig. 2).

DAISY ON LOMBARDY POPLAR.

INOCULATIONS OF APRIL 17, 1907 (SMITH).

Six trees of Lombardy poplar (*Populus fastigiata*) were inoculated with 5-day-old cultures, each receiving 30 pricks in groups of 5. Four trees were held as checks. The roots of all the trees were washed thoroughly in the laboratory before inoculating, and the trees were then planted in the greenhouse.

Result.—July 13, 1907: No tumors resulted from these inoculations. The areas punctured by the needle were still plainly visible on the yellow bark of the roots as little black patches.

INOCULATIONS OF MAY 26, 1908 (SMITH).

Three shoots of *Populus fastigiata* were inoculated (soft wood near the growing tip) with daisy organism from agar streak 4 days old.

Result.—June 1, 1908: Nothing definite.

November 16, 1908: No tumors have resulted. It is difficult to find where the needle entered. So far as these experiments go they tend to show that the Lombardy poplar is not susceptible to this disease, but not enough tests have been made.

DAISY ON COTTONWOOD.

INOCULATIONS OF APRIL 17, 1907 (SMITH).

Six trees of *Populus deltoides* were inoculated with 5-day-old cultures, each receiving 30 pricks in groups of 5. Four trees were held as checks. The roots of all the trees were washed thoroughly in the laboratory before inoculating, and the trees were then planted in the greenhouse.

Result.—July 13, 1907: No tumors have resulted from these inoculations, probably because the trees were too old to stand transplanting

well, i. e., the careless breaking of large roots. They were an inch or two in diameter at the base when inoculated and have made a slow growth. The areas punctured by the needle are still plainly visible on the yellow bark of the roots as little black patches.

DAISY ON ONION.

Inoculations of January 6, 1907 (Brown).

The bulbs (part above ground) and also the leaves of *Allium cepa* were inoculated by needle pricks with agar streak cultures 4 days old. Three checks were held.

Result.—February 1, 1907: No knots formed.

Inoculations of April 4, 1910 (Brown).

Eleven onion plants, growing slowly, were pricked in the bottom of the plateau from an agar streak culture of newest isolation.

Result.—June 24, 1910: All negative.

EXPERIMENTS WITH SCHIZOMYCETES FROM GALLS ON OTHER PLANTS.

In connection with our studies of the organism derived from the cultivated daisy, isolations from galls on other plants, together with pure-culture inoculations and cross-inoculations, were undertaken as follows:

HONEYSUCKLE ON DAISY.

Inoculations of April 14, 1908 (Smith).

Eight daisy plants were inoculated by needle pricks with agar streak cultures 4 days old of an organism plated out of an old knot on Japanese honeysuckle, found near Washington by Mr. W. A. Orton. The honeysuckle knots were about one-fourth inch in diameter. The knot from which these cultures were made was somewhat cracked open, and the plates came up with a variety of bacterial colonies, white and yellowish. A half dozen of these colonies approaching the daisy organism in appearance were selected, from which transfers were made.

Result.—June 1, 1908: No tumors. This means perhaps that the two colonies selected for these inoculations were not the right thing.

ARBUTUS ON DAISY.

Inoculations of November 2, 1909 (Brown).

Five young daisy plants were inoculated with agar cultures 5 days old plated from gall on *Arbutus unedo* sent from France. The daisy plants were of the lot obtained from a grower in Boston and were in splendid condition. Several checks were held.

Result.—March 26, 1910: No galls formed on any of the plants.

INOCULATIONS OF MAY 7, 1910 (BROWN).

Six terminal young flower shoots of slow-growing old daisy plants already bearing daisy galls on the main stem were selected for inoculation. Into these the Arbutus organism was pricked from young agar streaks.

Result.—June 25, 1910: All negative.

July 7: Same.

ARBUTUS ON SUGAR BEET.

INOCULATIONS OF NOVEMBER 8, 1909 (BROWN).

Sixteen young sugar beets growing in the open bed were inoculated with 2-day-old agar cultures of the Arbutus gall organism. Six checks were held.

Result.—December 20, 1909: Examined one row and found a gall three-fourths of an inch in diameter on one beet only. It was left to grow.

March 26, 1910: Reexamined plant with gall and found that it had rotted off.

June 10, 1910: Pulled up the beets and found one other with a gall 2 inches in diameter. Much of it had rotted off, but new parts were forming. This was photographed (Pl. XXIV, *A*).

COTTON ON DAISY.

INOCULATIONS OF NOVEMBER 16, 1909 (BROWN).

A dozen inoculations were made on young stems of plants in fine condition by needle pricks from agar cultures 5 days old. Three checks were held.

Result.—January 4, 1910: No galls formed.

INOCULATIONS OF APRIL 29, 1910 (BROWN).

Seven young shoots of the Queen Alexandra daisy were inoculated with 2-day-old agar cultures of the cotton-gall organism, using needle pricks. These plants were old but were putting out young flower shoots. They already bore large galls on the lower part of the stem as the result of earlier inoculations with daisy.

Result.—July 7, 1910: No galls resulted.

COTTON ON COTTON.

INOCULATIONS OF DECEMBER 1, 1909 (BROWN).

Nine seedling cotton plants about 6 inches tall were inoculated with 2-day-old cultures of the cotton-gall organism.[a] The soil was laid

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[a] When this organism was originally isolated there were pure cultures on 5 of the 8 plates poured.

back from the root and the crown inoculated by needle pricks. Three checks were held.

Result.—January 4, 1910: Four of the 9 plants had tiny galls. The checks were free. The plants had grown scarcely any since the time of inoculation.

COTTON ON SUGAR BEET.

INOCULATIONS OF NOVEMBER 11, 1909 (BROWN).

Nine young sugar beets were inoculated at the crown with 5-day-old agar cultures of the cotton-gall organism, using needle pricks. The beets were young and growing in the open bed.

Result.—January 4, 1910: No galls.

March 7: Plants were pulled up—no galls.

INOCULATIONS OF JULY 5, 1910 (BROWN).

Six young sugar beets were inoculated with 4-day-old agar cultures of the cotton-gall organism, using needle pricks.

Result.—July 18, 1910: No galls. The beets grew very slowly, owing to the excessive heat.

GRAPE ON DAISY.

INOCULATIONS OF MARCH 28, 1908 (SMITH).

Sixteen daisy plants were inoculated from slant agar cultures of March 25, the organism being derived from a tumor on grape occurring in this country. The plants had not yet branched and were inoculated in young and tender shoots about 6 to 8 inches from the ground.

Result.—June 1, 1908: They have given no distinct tumors, but a much more corky development than would have resulted from the needle punctures alone. The plants are now in blossom. These plants may have been cuttings taken from somewhat resistant tumor-bearing plants, or it may not have been the right organism, or, finally, colonies derived from an organism of weak virulence may have been used.

INOCULATIONS OF AUGUST 31, 1909 (BROWN).

Six young daisy plants of a strain that had never been inoculated with any gall organism were inoculated with grape-gall organism 4 days old (used also for inoculating sugar beet and grapevines). The plants were very small, still in 3-inch pots, and had very little soft tissue. Eight checks were held. The plants were repotted after inoculating.

Result.—October 13, 1909: Three of the 6 daisy plants had galls. These galls resembled the regular daisy gall and looked unlike those of grape, owing, perhaps, to different tissue reaction of the plants.

January 19, 1910: Photographed natural size (Pl. X, fig. 2).

April 6, 1910: Galls are 2½ inches in diameter and still growing (Pl. X, fig. 3).

GRAPE ON OPUNTIA.

Inoculations of June 30, 1910 (Brown).

Needle pricks from an agar streak 6 days old on one plant in one group of punctures.

Result.—October 21, 1910: Negative.

GRAPE ON GRAPE.

Inoculations of August 31, 1909 (Brown).

Galled grape stems of the Champion variety were sent in from Lawton, Mich., and agar plates were poured on August 23. A few colonies appeared in 3 days, transfers were made, and from these grapes and daisy plants were inoculated.

Four grapevines (variety not known) in poor condition (no young shoots being present) were inoculated, 3 to 5 stems on each, with 4-day-old agar cultures.

Result.—October 13, 1909: Two of the 4 plants inoculated were covered with the small warty protuberances characteristic of grape gall (Pl. X, fig. 1).

Inoculations of September 7, 1909 (Brown).

Inoculated 6 young grapevines, several stems each (Seedless Sultana variety), with the grape-gall organism, cultures 3 days old. The vines were in pots and in fairly good condition and were inoculated in the youngest parts. Two plants were held as checks.

Result.—September 25, 1909: All of the inoculated plants had galls of the typical grape-gall kind. The checks had no galls.

GRAPE ON ALMOND.

Inoculations of March 27, 1908 (Smith).

Three young shoots of almond were inoculated copiously by means of needle pricks from 48-hour slant agar cultures (same as used on daisy of this date which failed), the organism being derived from tumor on grape occurring in this country.

Result.—June 1, 1908: The shoots have grown much since the date of inoculation, but as yet have developed no tumors.

December, 1908: None appeared. Possibly wrong organism selected. Certainly there are sometimes noninfectious white schizomycetes in the galls which on agar are very difficult to distinguish from the right organism.

INOCULATIONS OF JUNE 28, 1910 (SMITH AND BROWN).

Eleven small seedling almonds and 1 grafted almond were inoculated on the crown by needle pricks from a young slant agar culture, not of the same origin as the preceding.

Result.—July 18, 1910: Galls are forming on the crowns of several of these plants.

July 29, 1910: Plants dug; 100 per cent infected. Galls occur only where inoculated and are from one-eighth to three-fourths inch in diameter (Pl. IX, fig. 3). The check plants, 2 pricked and 4 unpricked, are free from galls. The grafted plant is S. P. I. 24809, N. E. H. 257. The seedlings were grown from hard-shell California almonds cracked and germinated in sand, after removal of the shell, this spring in one of our houses, and all were free from natural infection.

GRAPE ON SUGAR BEET.

INOCULATIONS OF AUGUST 31, 1909 (BROWN).

Three sugar beets were inoculated with agar cultures 4 days old (same cultures used this date also on grape and daisy).

Result.—October 13, 1909: No galls formed on the beets.

INOCULATIONS OF MAY 7, 1910 (BROWN).

Twelve well-grown sugar beets standing in one row in a bed in the hothouse were inoculated on the upper part of the smooth, white root by means of needle pricks from an agar culture 1 day old.

Result.—June 23, 1910: Eleven plants contracted the disease at the place of inoculation. One failed; this was a small, slow-growing plant much like those inoculated in 1909. Two other plants in the row, being very small at the time of inoculation, were omitted, and these are now free from galls. The inoculated plants are also free except in the vicinity of the spot where they were inoculated. Other rows of beets in the same bed remained free except as inoculated. The largest tumors are 2 inches across. They resemble the grape gall in having numerous smaller nodules on the swollen surface. (Pl. XXIV, *B*.)

Remarks.—That these galls were produced by the visible bacteria inserted and not by some invisible hypothetical x transferred along with the bacteria from the original gall and unable to grow in our media but capable of inducing galls when inadvertently put back into the plant along with the bacteria, is indicated by the fact that the following eliminating transfers were made:

(1) Organism plated from grape gall, August, 1909.

(2) Subcultures to slant agar from single typical colonies.

(3) Transfers once a month to agar and beef bouillon for about 7 months to keep the bacteria alive.

(4) Poured plates from last bouillon transfer made a few days before the beets were inoculated.

(5) Agar subcultures from colonies on 4.

(6) Beets inoculated from one of 5.

ALFALFA ON DAISY.

INOCULATIONS OF JUNE 14, 1909 (BROWN).

Four young daisy shoots were inoculated with the alfalfa gall organism, using pure cultures 4 days old.

Result.—August 20, 1909: One of the shoots inoculated had a small gall about three-fourths of an inch in diameter. No good daisy plants were available at this time, and the experiment was never repeated.

ALFALFA ON ALFALFA.

INOCULATIONS OF JUNE 14, 1909 (BROWN).

Four young alfalfa cuttings were inoculated with 4-day-old agar streak cultures, the colonies for which were isolated June 1, 1909, from an alfalfa gall occurring in a field in Alabama. The fine roots were inoculated and then tied with a piece of cord, which was later replaced by a wire. This was to locate the galls should they form, and also so as not to mistake nitrogen-fixing nodules for them.

Result.—July 6, 1909: The plants were knocked out of the pots and examined. No galls were found.

August 20, 1909: Examined plants again and found the inoculations had taken on two-thirds of the roots marked by the wires. There were no distinct galls like the daisy gall, but hairlike projections on which a nodule different from the nitrogen-fixing nodule was formed and from which fine roots projected.

INOCULATIONS OF JUNE 16, 1909 (BROWN).

Inoculated 4 roots on each of 2 alfalfa plants with colonies on a plate poured June 10 (second isolation from southern alfalfa plants). These plants were old and pot-bound, but were taken from pots and repotted after inoculation. Each root was tied with a knot of strong cord.

Result.—July 6, 1909: Examined roots and found no galls.

August 20, 1909: Concluded plants were too old and had made too little growth for development of galls.

INOCULATIONS OF JULY 16, 1909 (BROWN).

Forty-eight seedling alfalfa plants were inoculated in the crown, in the roots, and also in the nitrogen-fixing nodules with 4-day-old agar cultures from second southern isolation, making 10 to 20 pricks in each plant. Twelve checks were held.

Result.—August 20, 1909: No indications of galls forming or of hairy-root outgrowths.

December 2, 1909: Twelve plants had formed crown-galls. The checks were free.

As it was always necessary to knock the plants from the pots, wash and examine the roots, they were more or less injured and set back in their development by this procedure. Possibly the seedlings were too young for best results.

Inoculations of September 7, 1909 (Brown).

Inoculated 3 pots of 1-year-old alfalfa plants with pure cultures 3 days old and 6 pots of young plants having taproots 3 mm. in diameter. The roots in both sets were inoculated at the crown. There were 4 plants in each pot, making 36 plants in all. Eight plants (2 pots) were held as checks.

Result.—December 2, 1909: Examined the plants and found one pot (4 plants) with very distinct and numerous galls on the roots; and two other pots (8 plants) containing plants with less numerous galls on the roots, making in all 12 plants with galls. Tiny, fine roots projected from these galls. The checks were free. Plates were poured from one of these galls to reisolate the organism. Some of these galls were photographed (Pl. XVI, fig. 2*a*), some preserved in alcohol, and some given to Mr. Kellerman to preserve.

First Inoculations of December 8, 1909 (Brown).

Gall colonies grew on the plates poured December 2 (see preceding) and inoculations were made into young seedling alfalfa plants to check the cultures.

Result.—May 11, 1910: All of the inoculated plants now show galls.

Second Inoculations of December 8, 1909 (Brown).

The plants used were several years old and in poor condition. The culture used was a subculture from a colony on a poured plate of December 2, 1909.

Result.—May 11, 1910: No galls.

ALFALFA ON PEACH.

Inoculations of January 27 and February 1, 1910 (Brown).

On January 27 six peach trees, which had just started to send out leaves, were inoculated at the crown by needle pricks with 1-day-old cultures of the alfalfa gall organism (first isolation). The trees were in pots in the greenhouse.

On February 1 eight young peach trees of the same lot as those inoculated January 27 were inoculated with 1-day-old cultures of the

alfalfa gall organism isolated from galls on alfalfa, produced in the greenhouse by inoculation.

Result.—June 25, 1910: No galls on either set. The trees were also examined before June, but no record was made of the date.

ALFALFA ON SUGAR BEET.

INOCULATIONS OF JUNE 14, 1909 (BROWN).

The same set of cultures used for inoculating alfalfa this date were used to inoculate 3 sugar beets in the open bed. The part of the root just below the surface of the soil was inoculated, using pure agar streak cultures 4 days old.

Result.—August 20, 1909: Two of the beets had large galls; diameter nearly 2 inches. One of the beets could not be found.

INOCULATIONS OF JULY 16, 1909 (BROWN).

Five young sugar beets in pots were inoculated at the crown with agar cultures 4 days old.

Result.—August 20, 1909: Galls had formed on 4 of the sugar beets. They were small, however, because the plants had become pot-bound and had grown very little.

On August 23 a photograph was made (Pl. VII, fig. 3). On the lower roots of this plant were also some small nematode galls.

PEACH ON DAISY.

INOCULATIONS OF DECEMBER 2, 1907 (BROWN).

Ten daisy plants were inoculated in the stem by needle pricks from agar colonies obtained by the poured-plate method from a peach gall November 26, 1907. The plants were in pots in the greenhouse and were in a good growing condition. They were inoculated near the tip.

The variety of daisy used was Queen Alexandra, 4 plants being new ones from a firm in Philadelphia, where the gall disease of daisy was unknown.

Result.—January 9, 1908: Each of the 10 inoculations gave tumors; 4 check plants remained free from infection, as did also the uninoculated parts of the infected plants.

INOCULATIONS OF DECEMBER 4, 1907 (SMITH).

Thirty-six plants of the white Paris daisy were inoculated with bacteria plated November 23 from the interior of a peach gall received from a nursery in Maryland. All the inoculations were made by needle punctures, making 5 or 6 pricks. The plants were 12 to 14 inches high and growing rapidly, so there was an abundance of soft

tissues for puncturing. The slime was rubbed over an internode, pricks were made through it, and the wounds were rubbed again with the platinum loop. Check punctures were made on the opposite side of each one of these shoots, and a little higher up, or else upon twin branches. The first 12 plants were inoculated from as many colonies. The remainder were inoculated from 4 slant agar cultures made December 2 from as many colonies on the same poured plate, the tubes having remained in the thermostat at 30° C. for two days, and the surface being covered with a copious growth. There was also an abundance of cloudy fluid in the bottom of the tubes and this fluid was pricked in very thoroughly. There were always a greater number of check pricks than of punctures with the infected needle. Usually 20 punctures were made with a sterile needle on each plant. All of the punctures were near the tops of the shoots in tender tissues, i. e., the ones most certain to give results.

Result.—January 14, 1908: All but 3 of these 36 plants (92 per cent) yielded distinct tumors in the inoculated part. None of the more than 600 check punctures on the same plants showed any tendency to form tumors.

May 15, 1908: Photographs were made (Pl. XIII, fig. 1).

November 16, 1908: About one-third of the daisies inoculated December 4 of last year are still living; the rest have died this summer. The largest tumors are 2 inches in diameter.[a]

In this connection the following notes on the origin and appearance of the bacteria used for these inoculations will be of interest. The crown-gall of the peach was scraped, washed, and the denuded surface further deadened by plunging into alcohol and then for five minutes in 1:1,000 mercuric chloride water. The interior of the knot was then entered by means of a sterile scalpel and scraped into sterile bouillon, from which the poured plates were then made. The scrapings from this tumor were thrown in considerable quantity into three different tubes of bouillon. Plates were poured from each one of these and also from bouillon dilutions of the same. The tubes which received the scrapings were marked A_1, B_1, C_1, and the dilutions were marked A_2, B_2, C_2. Apparently, not a great many living organisms were in the knot, and the dilutions did not yield satisfactory plates. The number of bacteria in the plates appeared to bear a constant relation to the amount of infectious material put in, i. e., those plates which were sown thickest gave the most colonies. The plates were incubated at room temperatures varying from 20° to 23° C. The plates were poured by Miss Florence Hedges, using +15 peptonized beef bouillon containing 1 per cent of agar. The

[a] The largest natural tumor observed on the daisy was on a root and measured 4¾ by 6 by 3 inches. Toumey figures much larger ones from the almond. (See also poplar, Pl. XXIII.)

knot was scraped, washed, sterilized, etc., by Dr. Smith. After 10 days at room temperature the plates gave the following results:

(A_1) *Two-millimeter loop:* This has been given about 75 colonies. These colonies are nearly all on the surface, i. e., they have been buried and have broken through. The largest surface colonies are now 4 mm. in diameter, the smallest ones are 1.5 mm. in diameter. They are circular in outline and uniform in appearance by transmitted light, except that the margins are a little clearer. The granulations in them are too fine to be visible with a Zeiss aplanatic lens magnifying six times. Nearly all of them have been buried colonies and show a darker, elliptical, triangular, or ragged buried central portion corresponding to the original buried colony. The margins are very sharply defined. The colonies are wet-shining on the surface, smooth. They do not seem, with the hand lens, to have any structure. They are not pink, nor greenish, nor yellow, but white by reflected light, and by transmitted light very slightly brownish. So far as can be determined with the hand lens, all the colonies in this plate are one thing. The buried ones are much smaller.

(A_1) *Needle inoculation:* This plate contains 8 colonies. They are of the same general appearance as the colonies in the other plates except that 1 marginal colony seems to be different, i. e., has a yellowish tinge.

(A_2) *Inoculated with a 2-mm. loop:* This plate contains nothing.

(B_1) *Two-millimeter loop:* This plate contains 56 colonies, of which 2 are mold spores, 1 is a thin-growing buried organism of uncertain nature, and the remainder are like those already described; circular, smooth, wet-shining, rather rounded-up surface colonies, slightly darker in the greater portion of their mass than at the extreme margin, which is sharp. These colonies have a darker center, corresponding to the buried growth from which they have arisen. There is a fine granulation in the colonies, but nothing distinct under the hand lens. The largest of them measures 6 mm. in diameter. The smallest surface ones measure about 2 mm. The colonies, like those in plate A_1, are distinctly rounded up from the margin to the center. This can be seen very well by looking sidewise through the plate across the top of the agar. The buried colonies are elliptical, pointed, or triangular.

(B_2) Inoculated with two 3-mm. loops: This plate contains 4 white colonies, 3 of which are typical; 1 has crenate margins and is an intruder.

(B_2) This inoculation, made with one 2-mm. loop, contains nothing.

(C_1) Two-mm. loop: This plate contains 44 colonies, of which 1 is a small intruding mold spore, and the others appear to be the same

thing and just like those colonies in plates made from A_1 and B_1. The surface colonies are round, smooth, wet-shining, white, rounded up from the margins, uniform in structure, except a little paler toward the edge, which is sharp. They have darker centers corresponding to the buried colonies from which they arose. So far as can be seen under the hand lens they have a uniform very fine granular structure. The largest of these colonies is 5 mm. in diameter and the smallest is 1.5 mm. The buried ones are elliptical, pointed, like those already described.

(C_1) Needle inoculation: This contains 10 colonies, all of which are alike and evidently the same organism as in A_1 and B_1. The largest surface colony is 6 mm. in diameter and the smallest one is about 1.3 mm. In their internal structure and their general elevation above the surface the colonies are precisely like those already described.

(C_2) Contains about a dozen colonies (thin, wide expansions), none of which appear to be like those already described. Most of them have lobed margins and are clearly some other organism. Probably infected in pouring.

(C_2) Needle inoculation: Contains one colony, which appears to be of the right sort.

The structure of the colonies in these plates under the miscroscope (Zeiss 16 mm. and No. 12 ocular) is precisely that of the daisy organism which I have just examined.

August 10: Photographs were made of Nos. 10 and 17 (Pl. XXV, fig. *C*.)

January 27, 1908: Alcoholic material was preserved and photos were made. Plates were also then poured from two of the knots.

February 3: The results obtained from the plates (seventh day) were as follows: (1) The plates from one knot were discarded because they contained yellow and pink colonies, i. e., saprophytes; (2) the set of plates from the other knot contained yellow colonies and white ones. The latter were most numerous and looked like what was inserted. In a very thinly sown plate the largest surface colonies were 8 mm. in diameter. They were perfectly circular, smooth, wet-shining, with sharp margins. The colonies were rather dense and nearly homogeneous to the naked eye, but under the hand lens they were seen to consist of a dense buried central spindle ringed by a clearer space, which was surrounded by a denser zone followed by a marginal clear zone. None of the zones were very sharply defined. The colonies were pure white, i. e., there was no yellow, pink, or greenish in them. In plates containing about 150 colonies the surface ones were circular, white, wet-shining, smooth, and rather dense, being 2 to 4 mm. in diameter. The buried colonies were spindle

shaped, sharply pointed, and most of them had broken through to the surface, while others were doing so. The only crystals in the plate were inside some of the yellow colonies. There were about 30 of these yellow colonies. The surface of the knot was probably not bathed in 1:1,000 mercuric chloride water long enough, i. e., only 10 seconds. Subcultures were made from 10 colonies on 2 of the thinnest sown plates, and 10 other similar white colonies on the plates were used to make the successful inoculations of February 3.

Inoculations of February 3, 1908 (Smith).

Ten daisy plants, Nos. 40 to 49, inclusive, were inoculated with the peach organism (originally plated from crown-gall of the peach, inoculated December 4 into daisy with production of tumors; then on January 27, plated from one of these tumors and now reinoculated into this group of daisies). The plants were each inoculated from a separate poured-plate colony.

Result.—June 1, 1908: Each of the 10 daisy plants finally developed a tumor (from one-fourth inch to over an inch in diameter) in the inoculated spot; but they were very slow to appear. They showed no tumors in any other place except No. 48, which developed a small tumor at the surface of the ground about a foot below the inoculated part. Photographs made (Pl. XIII, fig. 2).

Inoculations of March 11, 1908 (Smith).

Two daisy plants were inoculated with peach organism from colonies on poured plates of March 4. Two plants were held as checks.

Result.—June 1, 1908: No tumors; none on checks. These daisy plants were inoculated as checks on Wealthy apple, inoculated with the peach organism from the same poured plates, and their failure to produce tumors was due probably to small amount of inoculating material left after inoculating the apples (since the latter contracted the disease), or to the fact that they were inoculated directly from the plate, as would seem to be the case, from similar looking but really different colonies, or finally to the possibility of the daisy cuttings having been made from somewhat resistant (previously inoculated) stocks (p. 177).

PEACH ON OLIVE.

Inoculations of March 11, 1908 (Smith).

The tops of 2 tender shoots were inoculated with the peach organism from poured-plate colonies of March 4.

Result.—June 1, 1908: One of the inoculated shoots had grown 15 inches since the date of inoculation and the other one about 10 inches. No tumors.

This is in the same set of inoculations as that of the Wealthy apples, which took the disease (Pl. XII, fig. 2), and the 2 daisies which did not take it.

November 16, 1908: No tumors.

PEACH ON PHLOX.

Inoculations of May 18, 1909 (Brown).

Young annual phlox plants in pots just starting to bloom were inoculated near the tips of the stems by needle pricks with agar cultures 4 days old of the peach-gall organism (isolated February 29, 1908). Ten plants were inoculated and 4 were held as checks.

Result.—June 8, 1909: No indications of galls could be seen.

July 14: Examined again—no galls. The cultures may have lost their virulence.

PEACH ON VERBENA.

Inoculations of May 18, 1909 (Brown).

Young verbena plants growing in pots were inoculated with 4-day-old agar cultures of the peach-gall organism (isolated February 29, 1908), the stems being pricked at the tips, at the base, and midway between. Ten plants were inoculated and 4 were held as checks.

Result.—July 14: No indication of galls.

PEACH ON GRAPE.

Inoculations of June 24, 1910 (Smith and Brown).

The terminal part of 2 green shoots of *Vitis vinifera* was inoculated by needle pricks from a 2-day-old agar streak culture of the crown-gall organism from the peach (isolated February 29, 1908).

Result.—July 18, 1910: Doubtful; only slight prominences.

October 31, 1910: Nothing on one; very tiny elevations in needle pricks on the other; no true galls. Organism had probably lost virulence.

PEACH ON IMPATIENS.

Inoculations of June 24, 1910 (Smith and Brown).

One pink-flowered plant on 3 shoots and 1 white-flowered plant on 2 shoots were inoculated by needle pricks from 48-hour-old agar streaks of the crown-gall of peach organism (isolated February 29, 1908). The stems were soft.

Result.—October 21, 1910: All negative.

PEACH ON PELARGONIUM.

Inoculations of October 13, 1908 (Smith).

Two vigorous-growing shoots on each of 2 plants of a common red-flowered *Pelargonium zonale* were inoculated by needle pricks from 3-day-old agar streak cultures of the peach organism after it had been passed through red raspberry.

Result.—November 16, 1908: Each of the 4 shoots bore a small whitish corky-looking tumor where the needle entered, i. e., about 1 sq. cm. was raised above the surface of the stem 3 mm. or more.

December 9, 1908: Two of the shoots were photographed and the material then fixed in Carnoy's solution for sections.

January 18, 1909: The other 2 shoots were brought in and photographed (Pl. XIV). These shoots were still leafy and vigorous. The tumors were more than an inch in diameter, but did not seem to have done the plants any injury, i. e., the foliage above the gall was not yellow nor dwarfed.

PEACH ON PEACH.

Inoculations of December 5, 1907 (Brown).

Six young peach trees were inoculated with the peach-gall organism, 25 needle punctures being made in groups of 5 along the root, beginning at the crown. Agar streak cultures 3 days old made directly from the plate colonies were used. The inoculations were made in the laboratory and the plants set out in pots in the greenhouse. Two controls were held, the needle pricks being the same in number and position.

For comparison 6 peach trees were also inoculated with the daisy organism, using agar cultures of the same age, and making the punctures in the same way, in groups of 5 on the root, beginning at the crown.

Result.—January 8, 1908: Four of the 6 trees inoculated with the peach knot organism had decided galls one-third to one-half inch in diameter; and 4 of the 6 trees inoculated with the daisy organism had galls about the same size.

January 15: A photograph was made of the peach (Pl. XI, fig. 1).

February 14, 1908: All the trees inoculated with the peach gall organism had galls, while galls had formed on only 4 of the 6 inoculated with the daisy organism. The galls were 1 to 2 inches in diameter and alike on each tree. All the galls occurred at the crown or just below it, in no case on the deeper inoculated roots, nor were there any galls at other than the inoculated places. The 2 check plants remained free from galls.

INOCULATIONS OF JANUARY 13, 1908 (SMITH).

One hundred and thirteen seedling peach trees 1 year old were received through Mr. Corbett from the Arlington Experimental Farm. All of them were in good condition except 5, which were rejected because of borers. All were free from nematode galls and also from crown galls. Twelve were given to Doctor Townsend. (See *Rose on Peach*, p. 76.) The remaining 96 were divided into 2 lots. The dirt was first thoroughly washed from the roots; then the roots were shortened a little and the tops pruned back, so that they could be planted in 10-inch pots in the hothouse. The 2 groups were then treated as follows:

(1) Thirty-six trees were held as absolute checks, 15 pricks in groups of 5 being made with a sterile needle on the roots of each tree. The check pricks on all the plants were made before any inoculations were undertaken.

(2) This group of 60 trees is like group 1, except that opposite the 15 check needle pricks (3 groups of 5 each) 15 infected pricks (introducing a pure culture of the organism from crown gall of the peach) were made on the crown and roots in groups of 5 each. The side on which the check pricks were made was marked by cutting a sliver out of the bark of the stem, above the crown, with a sharp knife. A good deal of the white slime was put on the roots in making these inoculations (in most cases on the main root, rarely on side roots), and as they were planted within an hour or two of inoculation, there was a possibility of the slime infecting some of the check pricks by finding its way to the other side of the root; with a view to lessen this possibility, instructions were given the gardener to withhold water until the following day, if this could be done without injury to the trees. They were planted in good greenhouse soil.

Result.—March 4, 1908: The peach trees planted January 13 were pulled up and examined:

(1) Group of 36 check plants: 33 absolutely sound, 3 with slightly enlarged callus about as many knife wounds, none with galls. The trees received 540 stabs, all of which healed normally.

(2) Group of 60 inoculated plants: 55 with galls; 5 free. The 55 plants bore 127 galls—with very few exceptions, only where inoculated. The few exceptions are minute galls in the vicinity of the pricks on smaller accidentally injured roots. The 900 check pricks remained free from galls. The best galls were photographed (Pl. XI, fig. 3) and put into alcohol. None were very large, but sufficient for the time concerned, i. e., less than 2 months—the largest one-half to five-eighths inch in diameter.

Had the trees been ready to leaf out when planted it is probable that the percentage of infections would have been 100 instead of 92.

A greater number of the upper sets of pricks failed than of those farther down on the root. (By "upper" is meant near the crown.)

The surface of selected washed galls was sterilized three minutes in mercuric chlorid water (1:1,000), portions from their interior removed with sterile knives, mashed in beef bouillon, and plates poured—one set by Miss Florence Hedges and another by Miss Lucia McCulloch.

March 7, 1908: Each set of plates yielded many colonies of the right organism.

Inoculations of March 24, 1908 (Brown).

On February 29 an organism was plated out of the interior of one of the galls (peach strain) obtained by the inoculations of December 5, 1907, and on March 24 inoculations were made in the greenhouse on 10 healthy peach trees to determine whether or not this was the crown-gall organism, i. e., the same schizomycete as that inserted. Four-day-old agar streak cultures were used—the first subculture from the poured-plate colonies. The inoculations were made at the crown by means of needle pricks. The trees had been moved in recently from a cold frame. Five of the trees had developed foliage; 5 others were just beginning to show foliage. Four checks were held, punctures being made in the same way as on the inoculated plants.

Result.—June 2, 1908: Galls 1 inch to 2½ inches in diameter were found on 5 of the inoculated trees. On one other tree a gall had formed and then rotted off. Three trees showed no indication of galls, but the roots were abnormal, i. e., there were many fibrous roots. The tenth inoculated tree was missing. The 4 check plants remained free from galls. On the inoculated plants the galls were restricted to the inoculated parts.

August 10: Photograph made.

All further inoculations with the peach gall organism so far as made by Miss Brown were with this strain plated from one of the galls produced by inoculation.

PEACH ON APPLE.

Inoculations of January 16, 1908 (Brown).

Six young apple trees from the Arlington Experimental Farm were inoculated with agar streak cultures 1 day old, the fourth subculture. Fifteen needle pricks were made on the main root of each plant. Four check plants were held. The inoculations were made in the laboratory and the trees set out in pots in the greenhouse. The trees (variety Wealthy) were not in good condition and were dormant. It is important to keep these facts in mind.

Result.—April 7, 1908: No trace of a knot on any tree.

INOCULATIONS OF JANUARY 23, 1908 (BROWN).

Five young Ben Davis apple trees, and 5 young Wealthy apple trees were inoculated. Two Ben Davis and 4 Wealthy were held as controls.

A number of trees were discarded at the time of inoculation because of the fibrous condition of the roots, and all were more or less suspicious because of the soil in which they had grown. The cultures used for these inoculations were 2-day-old agar streaks, the fifth subculture from plate colonies. From 15 to 25 needle pricks were made on the main root of each plant on one side only, and the side inoculated was indicated by a notch on the stem. The trees were planted in pots in the greenhouse.

Result.—June 2, 1908: Two of the Wealthy apple trees had well-developed knots on the inoculated side. Three were without knots. Only 3 of the 5 inoculated Ben Davis trees could be found, and all of these had knots. These knots were on the punctured side of 2 of the trees; but no positive conclusion can be drawn because of the behavior of the controls. Of the 4 Wealthy used for checks 3 bore knots and 1 was doubtful. Of the 2 Ben Davis 1 had a knot and 1 was free.

As the controls bore knots, and in places where there were no needle pricks, the conclusion was drawn that some of the trees at least were infected in the field.

INOCULATIONS OF MARCH 11, 1908 (SMITH).

Three Wealthy apple trees were inoculated (1 in two places on a top shoot and in one place on the base of a shoot at a considerable distance above ground; the other 2 in the top shoots only) with the peach-gall organism from poured-plate colonies of March 4—i. e., derived from the interior of a crown gall on peach produced by a pure-culture inoculation.

Result.—June 1, 1908: The top shoot of plant 1 has given 2 well-developed small tumors, the larger one round and about five-eighths inch in diameter. The inoculation on the base of the shoot has resulted in 4 distinct small tumors, each about 4 mm. high and 2 to 5 mm. broad.

Plant 2 was inoculated in the top shoot in two places. In one place it has given a tumor about 2 mm. high and about the same diameter, and in the other place it has given 3 separate tumors, each about 2 mm. high and 2 mm. in diameter.

Plant 3 was inoculated at the base of two top shoots, each one of which has given a group of small tumors 2 mm. in diameter and 2 mm. high. One of them has 4 of these tumors and the other has 3. The development of these galls has been very slow. There are no other tumors on the plant.

August 14, 1908: All the larger galls were removed. They are now 2 inches in diameter.

November 16, 1908: The remaining tumors have grown a great deal and have partly decayed.

April 2, 1910: The hard galls on tree No. 2 were photographed (Pl. XII, fig. 2), the tree repotted, and the galls wrapped in sphagnum to see if roots would develop from them. The buds on the tree are just opening.

June 25, 1910: No roots have yet formed on peach gall growing on apple under wet sphagnum, but the gall has begun to make new growth in places.

October 13, 1910: The sphagnum was removed and the galled portion of the tree brought in and examined. A considerable area of finely warted new tissue had formed and some parts of the gall had given rise to small roots, but they were not of the hairy-root type.

The peach organism was recovered from this gall by means of poured plates.

Inoculations of May 10, 1908 (Brown).

Thirteen seedling apple trees, ranging from 6 to 12 inches in height and growing in pots in the greenhouse, were inoculated with the peach gall organism as follows: Seven at the crown and also on the stem; 6 at the crown only. Agar streak cultures 4 days old were used for the inoculations. Four check plants were held. These were punctured with a sterile needle on crown and stem.

Result.—September 2, 1908: A gall 2 inches in diameter on the stem of one tree; a gall one-half inch in diameter on the stem of another tree. Galls had also formed at the crown of 3 other trees. One of these was $1\frac{1}{2}$ inches in diameter. Two of the trees inoculated on stem and crown did not make any growth. Of the trees which were inoculated at the crown only, 2 died and 4 did not make any growth, consequently no tumors formed. In this experiment we may claim 100 per cent of infections if we exclude the 8 trees which did not grow.

There were no galls on the check plants.

Inoculations of May 22, 1908 (Townsend).

Thirty-five apple seedlings (variety, Kansas) were inoculated by needle puncture on the crown just above ground, using agar streak cultures of peach organism 3 days old.

Thirty-five trees of the same kind were punctured in the same way with a sterile needle for control.

Eighteen apple seedlings (variety, Virginia) were inoculated in the same way.

Nine trees of the same kind were punctured for control.

Result.—November 24, 1908: Of the inoculated Kansas, 10 trees made no growth, 12 were missing, and 1 out of the remaining 12 bore a knot. Of the supposed controls, 5 made no growth, 14 were missing, 6 bore knots, and 10 were free.

Of the inoculated Virginia only 4 bore knots; 5 only of the controls were found. On these there were no knots.

All of these trees were grown on the Arlington Experimental Farm. They were dug, washed, inoculated, and replanted at Arlington. Doctor Townsend superintended the washing and inoculation, but not the replanting. The soil in which the trees had grown, and in which they were replanted, was believed to be free from the gall organism. The appearance of galls on the trees marked as checks was attributed to the workman's having mixed checks and inoculated trees at the time of replanting. The failure of this experiment emphasizes the necessity of safeguarding every step of an experiment.

PEACH ON RED RASPBERRY.

Inoculations of May 20, 1908 (Brown).

Nine young red raspberry bushes in a good growing condition in pots in the greenhouse were inoculated with agar streak cultures 2 days old. Seven plants were inoculated on the crown and on the stem. Two plants were inoculated on the crown only. Four checks were pricked on crown and stem.

Result.—June 12, 1908: One hundred per cent of infections. Four of the 7 plants inoculated on crown and stem bore knots on both crown and stem, while the other 3 had knots at the crown only; of the 2 plants inoculated only on the crown, both bore knots. Those plants inoculated on the stem received 2 to 3 groups of punctures, each one yielding a gall.

No galls formed on the 4 checks.

PEACH ON BLACK RASPBERRY.

Inoculations of May 19, 21, 22, 1908 (Brown).

Thirty-four plants were inoculated, as follows:

May 19. Ten young black raspberry bushes in a good growing condition in pots in the greenhouse were inoculated with agar streak cultures 8 days old; 6 plants, both on the crown and on the stem; 4 plants, on the crown only. Four checks, punctured on both crown and stem, were held.

May 21. Twelve similar plants were inoculated at the crown and on the stem with agar streak cultures 3 days old.

May 22. Twelve similar plants were inoculated on the crown only with agar streak cultures 2 days old.

Results.—June 12, 1908: Plants inoculated May 19: All the inoculated places had galls except 1. The exception was 1 of the 4 plants inoculated only on the crown.

All plants inoculated May 21 had knots on both root and stem, except 1 plant which had a knot on the stem only.

All plants inoculated May 22 had knots.

Total number of inoculated plants 34, of which 33 bore galls. In most cases there were several groups of punctures on the stem, each of which yielded a gall.

The 4 check plants remained free from galls.

April, 1909: The above galls rotted away and in 8 or 10 instances new galls developed from their margins.

PEACH ON ROSE.

Inoculations of January 15, 1908 (Brown).

Six rose bushes (variety Killarney) were inoculated at the crown by needle pricks from 1-day-old agar streak cultures, second subcultures from the poured-plate colonies. The plants used were in pots in the greenhouse. The soil was laid back carefully, and, after puncturing, the inoculated places were covered with moist cotton.

Result.—April 17, 1908: A gall one-third inch in diameter on one plant. (Pl. II, fig. 1.) No trace of an enlargement on any other. The plants were not growing rapidly.

Inoculations of June 27, 1910 (Smith and Brown).

Twelve shoots of rooted cuttings of as many rose plants (variety, Killarney) were inoculated by needle pricks from agar streak cultures 3 days old, puncturing into the softest wood.

Result.—October 21, 1910: All negative. The plants grew slowly. The organism had probably lost virulence.

PEACH ON MAGNOLIA.

Inoculations of June 24, 1910 (Smith).

The terminal part of 4 young rapidly growing shoots of a broad-leaved magnolia (*M. acuminata?*) were inoculated by needle pricks from a 2-day-old agar streak culture of the crown-gall organism isolated (February 29, 1908) from the peach.

Result.—July 30, 1910: Negative; suspect that organism has lost its virulence.

PEACH ON PEONIA.

INOCULATIONS OF MAY 6, 1909 (SMITH AND BROWN).

Four roots of *Peonia officinalis* were inoculated with peach gall organism—culture 2 days old (isolation of 1908). Leaf buds were just starting out of the root stocks, and inoculations were made at the base of these, also on the roots themselves. Three checks were held.

Result.—September 2, 1909: The plants were knocked out of the pots and examined carefully. No galls were found in those inoculated. We were led to make these inoculations because Dr. Whetzel reported finding root-galls on peonia in New York.

PEACH ON SUGAR BEET.

INOCULATIONS OF MARCH 11, 1908 (SMITH).

Five sugar beets were inoculated by needle pricks on the crown with an agar subculture of an organism isolated from a crown-gall on peach and previously passed twice though the peach with the production of tumors.

Result.—May 4, 1908: Each one of the 5 plants contracted the disease at the point of inoculation and not elsewhere (Pl. VI, fig. 2).

PEACH ON HOP.

INOCULATIONS OF JUNE 10, 1908 (BROWN).

Six young hop plants from seed grown in sterile soil in pots in the greenhouse were inoculated by needle pricks at the crown with agar streak cultures 4 days old. Two plants kept as checks were punctured in the same way at the crown.

Result.—June 30, 1908: 100 per cent of infections. Knots three-fourths inch to an inch in diameter had developed on each one of the 6 inoculated plants. These knots were white and grew more quickly than those on the peach. The checks remained free.

PEACH ON RED OAK.

INOCULATIONS OF MAY 20, 1908 (BROWN).

Young seedling red oak trees about 6 inches tall were inoculated on the crown and on the stem with agar streak cultures 2 days old. Eleven trees were inoculated; 4 were held as checks.

Result.—September 2, 1908: The trees had not made any noteworthy growth, but a small knot or knobby outgrowth was present on one of the inoculated stems. The checks remained free.

PEACH ON PERSIAN WALNUT.

INOCULATIONS OF OCTOBER 13, 1908 (SMITH).

Two shoots of *Juglans regia* were inoculated by needle pricks with agar streak cultures of *Bacterium tumefaciens*, plated by Miss Brown from knots on red raspberry, which were produced by inoculating with pure cultures plated out of peach crown-gall.

These agar cultures were streaked on the 10th of October. The two streaks are copious, somewhat raised up from the surface, smooth, and wet-shining. Under the hand lens the lower part of the streak has in certain lights very fine irregularities on its surface, not noticeable to the naked eye. The color of the slime is gray-white. The spread away from the needle track is considerable. At the top it extends to either side a distance of 2 mm.; toward the base it extends to either side of the needle track a distance of 5 mm. There is a slight amount of water in the V, and this also is filled with the gray slime. The edge of the track is slightly undulatory.

The walnut shoots were hard when inoculated, so no results were anticipated. The plants were in a hothouse.

Result.—No tumors developed. This negative result was attributed to the fact above mentioned, i. e., that the shoots had ceased to elongate and were hard when inoculated, so that the bacteria were inserted into slow-growing tissues. This is the more likely because inoculations made the same day on Pelargonium gave positive results.

INOCULATIONS OF JUNE 24, 1910 (SMITH).

Three green shoots of *Juglans regia* var. *pendula* were inoculated in the softer terminal portion by needle pricks from a 2-day-old agar streak culture of the crown-gall organism derived from the peach (isolated in 1908). The shoots had reached nearly their definite length and were, therefore, less satisfactory than they would have been at the beginning of the month.

Result.—August 15, 1910: No result. Possibly the organism is losing virulence.

PEACH ON TRADESCANTIA.

INOCULATIONS OF MAY 7, 1909 (BROWN).

Six growing stems of Tradescantia were inoculated by needle pricks with 3-day-old agar cultures of the peach-gall organism (isolation of 1908). Each stem was punctured 15 to 20 times. Two stems were punctured with a sterile needle for checks.

Result.—July 14, 1909: No trace of gall formation.

ROSE ON DAISY.

Isolation of Organisms.

On December 10, 1907, 2 rose bushes were found in the propagating greenhouse of the Department of Agriculture with galls 2 inches in diameter on the root below the graft. Plates were poured from the soundest part of one of these galls after proper scrubbing and surface sterilization, i. e., the scrubbed gall was pared with a sterile knife and small selected pieces designed for cultures plunged for about 2 seconds into mercuric-chlorid water (1:1,000), rinsed in sterile water, and crushed in sterile bouillon for the plates. In three days the typical gall colonies appeared.

Inoculations of March 18, 1908 (Brown).

Eight inoculations were made with rose A on daisy plants, 4 on a large yellow-flowered variety and 4 on the Queen Alexandra. One check.

Result.—March 31, 1908: Elevations at places inoculated indicate the beginning of knots.

April 25, 1908: Knots had formed but were quite small. They had not developed as rapidly as those due to the regular daisy-knot organism. On the rambler rose these same cultures produced no galls.

Inoculations of March 21, 1909 (Brown).

Three daisy plants of the Queen Alexandra variety were inoculated by needle pricks with 5-day-old slant agar cultures from the rose gall. Each plant was inoculated on from 3 to 5 shoots. Two plants were held as checks. These plants were growing better than those previously mentioned.

Result.—April 3, 1909: Small galls had formed at half of the inoculated places. The checks remained free.

September 13, 1909: The galls grew slowly, as shown in the photograph made on this date (Pl. VII, fig. 2).

ROSE ON ROSE.

Inoculations of December 17, 1907.

Twelve Killarney rosebushes were inoculated by needle pricks at the crown with agar slant cultures 4 days old, the first subculture from poured-plate colonies made December 10. Each inoculated place was covered with a small piece of moist cotton. The crowns of 3 other rosebushes were punctured with a sterile needle for checks.

Result.—January 8, 1908: Small, white, knobbed prominences projected from the dark-colored root about a quarter of an inch on 2 of

the 12 inoculated plants. These knots were under the pieces of cotton, placed over the punctures, so there was no mistaking the infection. The checks had no knots. These roses were not making a rapid growth.

Remarks.—The rose is rather difficult to infect. Probably if one knew just the right age and stage of growth infection might not be difficult, since some varieties of rose are very liable to contract this disease in hothouse culture, the Killarney in our experience being one of them.

Inoculations of January 15, 1908.

Twelve Killarney rosebushes were inoculated with agar streak cultures 1 day old. The soil was laid back from the crown of the root, and the root washed carefully with sterile water before inoculating. Fifteen needle pricks were made on each root. Each inoculated spot was covered with a piece of moist cotton.

Result.—February 3, 1908: Only 1 root had a gall.

April 17, 1908: The entire lot was again examined carefully, and but 1 other gall found. This one was taken to the laboratory and the organism was obtained from it by poured agar plates.

Inoculations of March 18, 1908.

Six young, healthy Rambler rosebushes were inoculated by needle pricks with agar streak cultures 2 days old. The plants were taken from the pots, the soil removed, but not washed, so that none of the fine rootlets were broken off.

Result.—April 6, 1908: The plants were taken from the pots and examined but no knots were found. The growth had been slow. (For checks, see *Rose on Daisy*, p. 75.)

Remarks.—Of 30 rose plants inoculated only 4 contracted the disease.

ROSE ON PEACH.

Inoculations of January 14, 1908 (Townsend and Brown).

Eight young peach trees were inoculated at the crown with slant agar cultures 1 day old. Twenty-five punctures were made in each tree in groups of 5. Four trees were held as checks. The work was done in the laboratory and the trees planted immediately afterward in pots in the greenhouse. The trees were dormant at the time of inoculation and probably remained so long enough to interfere with the infection.

Result.—April 6, 1908: The trees were taken from the pots, washed, and carefully examined. No galls were found on any of the trees.

Inoculations of May 9, 1909 (Smith and Brown).

Twenty-five peach trees dug up and brought over from the Arlington Experimental Farm May 8 were inoculated and planted out. The trees were rather leafy, and through an oversight the young foliage was not removed from them until 10 a. m. May 9, so they suffered considerably from transpiration. Ten of these trees were pricked with a sterile needle for checks, 15 or 20 pricks each in groups of 5, and the 15 remaining were inoculated with 1-day-old and 3-day-old agar streak cultures of the rose organism, which had been on culture media since the fall of 1908. The inoculations were made by means of needle pricks in groups on both sides of the main root in the yellow bark. After the punctures were made the plants were set out in 10-inch pots. Ordinarily the plants would not have been watered immediately after inoculation, but they had suffered so much from loss of water overnight that directions were given the gardener to water them carefully as soon as he had finished potting them.

Result.—September 3: Shook trees from pots, washed and examined roots; no galls on inoculated or check plants; 5 inoculated trees and 5 check trees were dead.

These trees were set back in their development by stripping the leaves in May, but this is scarcely sufficient to account for the non-infection. The rose-gall organism does not cross-inoculate readily.

ROSE ON APPLE.

Inoculations of January 23, 1908 (Brown).

Six apple trees were inoculated with agar streak cultures 2 days old. Punctures were made at 3 different places on each tree—at the crown, above the crown on the stem, and near the end of the shoot. Four controls were held. The variety of apple used was the Wealthy. The trees were dormant.

Result.—June 2, 1908: The trees were taken out of the pots, washed, and examined carefully. No knots were found. Here again possibly the dormant condition interfered with the infection.

ROSE ON SUGAR BEET.

Inoculations of December 3, 1908 (Brown).

Seven small sugar beets were inoculated by needle pricks just below the surface of the soil with agar streak cultures 7 days old. Three checks were made. The house was cold, and the plants were making a slow growth. When pulled up they were scarcely larger than when inoculated.

Result.—December 22, 1908: The 7 inoculated plants were examined carefully and but 1 gall found (Pl. II, fig. 6). It was three-fourths inch in diameter and regular in shape. The checks were examined and no galls found on them.

RASPBERRY ON DAISY.

INOCULATIONS OF JULY 9, 1907 (SMITH).

Four daisy plants were inoculated from the white colonies on Petri-dish poured plates of July 2. These colonies were plated out of a small growing tumor on the root of a red raspberry plant taken from one of our houses, the same being in all probability a natural infection. Most of the daisy plants inoculated had several branches, and each branch was inoculated toward the top in as soft tissue as could be found. The plants had been neglected, and the wood was rather hard for the purposes of inoculation.

Result.—Negative.

QUINCE ON DAISY.

ISOLATION OF ORGANISMS.

The galls on quince trees are very unlike those of apple, peach, chestnut, etc.—i. e., they are warty outgrowths massed together, rather than galls of the ordinary type.

Isolations were undertaken December 23, 1908.

The material for this work was sent from California by Mr. Ballard, and when it arrived it was very much dried out. The galled stems were soaked overnight and the plates poured from the softest part of the material after proper scrubbing and surface sterilization. Six days afterward gall colonies appeared on the plates.

INOCULATIONS OF JANUARY 27, 1909 (BROWN).

Inoculations were made into the stems of 8 daisy plants of both yellow and white varieties (4 of each) with agar streak cultures 2 days old, the first subculture from poured-plate colonies. Three checks of the white variety were held. All were old plants growing slowly.

Result.—February 11, 1909: Small knobs were present on 2 of the white daisies at the point of inoculation, but no indication of any on the yellow variety. There were no outgrowths on the check plants.

INOCULATIONS OF MARCH 21, 1909 (BROWN).

Several daisy plants of the Queen Alexandra variety were inoculated by needle pricks with agar streak cultures 5 days old. Nine different shoots were punctured. Two plants were also held as checks, several shoots being punctured.

Result.—April 3, 1909: Gall formation had begun to show on two-thirds of the inoculated places. The appearance was rather warty and not like the beginning of an apple or peach gall. The checks remained free.

QUINCE ON QUINCE.

Inoculations of May 14, 1909 (Brown).

Rooted cuttings of quince, from which the leaves had been pulled off and the stems trimmed back, were scrubbed well and then inoculated. The stem and the part of the stem from which roots were growing (it could scarcely be called crown on these cuttings) were inoculated. Both nodes and internodes on the upper stem were inoculated. From 30 to 50 punctures were made on each cutting. The side inoculated was indicated by a notch in the bark. Eight cuttings were inoculated and 6 checks were held.

Result.—June 15, 1909: No indication of galls.

July 13: Still no galls.

November 28, 1910: All negative.

Inoculations of May 21, 1909 (Brown).

Some of the lot of cuttings received May 14, 1909, had been planted without inoculating and were now starting to send out buds. Three trees were inoculated on the stems by needle pricks with 2-day-old agar cultures. Nodes where leaf buds were starting and also internodes were inoculated. Each stem received at least 30 punctures. The same number of trees were inoculated with the hairy-root organism in the same manner. (See *Hairy root on quince trees*, p. 103.)

Result.—September 3, 1909: Examined the trees and found no trace of galls. The quince trees in the greenhouse seemed to grow very little, for no change had taken place in the size of the young stems inoculated.

November 28, 1910: Still no galls. Trees grew more than last year.

Inoculations of March 9, 1910 (Brown).

Reinoculated 7 of the quince trees which were inoculated last May. They seemed to be in a good growing condition. Two-day-old agar cultures of the quince-gall organism were used and only young shoots were punctured—6 to 8 shoots on each plant.

Result.—June 24, 1910: No galls formed.

November 28, 1910: Nothing.

QUINCE ON SUGAR BEET.

Inoculations of February 26, 1909 (Brown).

Seven small sugar beets were inoculated just below the surface of the ground with 3-day-old agar streak cultures, the second subculture from the poured-plate colonies. Two checks were made.

Result.—March 31, 1909: No growth of the beets had taken place since the time of inoculation and no outgrowths were found, either on the inoculated plants or on the checks. The absence of galls should be ascribed probably either to a defective culture or to slow development of the beets rather than to any special resistance.

Inoculations of July 2, 1910 (Brown).

Four inoculations were made on middle-sized plants, and 12 on younger plants growing in a bed. All were made on the upper part of the root by needle pricks, using young agar cultures.

Result.—July 18, 1910: All negative. Here again the plants were making a very slow growth, owing to the excessive heat. Whether this second failure should be ascribed to the bad condition of the host plants or to the character of the culture must be left an unsettled point. Its cultural characters were unlike those of the cultures of proved pathogenic power (daisy, hop, peach, grape, poplar).

BEET ON DAISY.

Inoculations of April 26, 1910 (Brown).

Four terminal shoots were inoculated on old slow-growing daisy plants already bearing daisy galls on the main stem. A 4-day-old culture of an organism from the sugar beet on agar was used, and this was inserted by needle pricks.

Result.—June 25, 1910: Three shoots negative. The fourth bears in the pricked part one small gall about as large as a sweet-pea seed.

BEET ON ALMOND.

Inoculations of July 30, 1910 (Brown).

These almonds were part of the lot used for the grape inoculations of 1910, i. e., young grafted stocks. Four plants were inoculated by needle pricks and 2 were held as checks.

Result.—October 22, 1910: All negative.

BEET ON BEET.

Inoculations of June 27, 1910 (Brown).

Ten half-grown plants were inoculated on the upper part of the root by needle pricks from a young agar culture.

Result.—July 18, 1910: All negative. Weather hot and beets making a very slow growth.

Remarks.—The foregoing 3 experiments were made in all probability with the wrong organism, as shown by subsequent tests on culture media. The organism used had a slightly pinkish growth on agar. (See *Cultural characters*, p. 108.)

ADDITIONAL EXPERIMENTS WITH SUGAR BEETS.

Isolation of Organisms.

In November, 1910—i. e., since the foregoing paragraphs were written—additional galled sugar beets were obtained (1) from Colorado, (2) from Kansas, (3) from Michigan, (4) from Arlington Experimental Farm in Virginia, and (5) from the State of Washington.

The material from Colorado and Kansas was not satisfactory for reasons stated on page 194. From the Michigan material what was supposed to be the gall organism was obtained twice (two sets of plates from one gall). From the Washington material a gall-like organism was obtained once. From the Arlington material it was obtained 5 times (4 different beets). Two of the above tests (Arlington) were quantitative tests. The first one was lost so far as quantitative results are concerned, either because the sand used in grinding was not sterile or because the surface of the gall was simply scraped and then washed repeatedly in sterile waters without subjecting the surface to heat or germicidal solutions. The second test, made with greater care as to sand and surface sterilization, yielded the results hereafter detailed; but before these are given it will be well to put before the reader the technique employed.

On November 15, 1910, a sound, medium-sized sugar beet was selected. It bears one tumor free from decay or cracks. It is rounded oblong, attached by a rather broad base, and free from surface irregularities, being covered by a thin secondary cork layer (wound cork). The beet was washed clean in tap water, plunged into alcohol until free from air bubbles, then into 1:1,000 mercuric chloride-water for 20 minutes. During this period the tumor was scraped gently with a knife to remove all the cork without injury to the deeper tissues. With the point of the knife a few tiny black specks extending into the white surface of the gall a very little deeper

than the average cork layer were also removed. Once or twice the denuded surface was also rubbed gently under the disinfectant with the finger tip. Great care was taken not to wound the deeper tissues. At the end of the 20 minutes the beet was rinsed quickly in a large volume of sterile water, then wrapped in sterile paper and put away over night. Only that mercuric chloride lying on the surface was washed away, not that absorbed into the superficial layers of the tumor.

On November 16 washed pure white sand (for grinding the tissue) was dry heated in the oven one hour at 240° to 250° C. The Wedgwood mortar and glass pestle and the distilled water used were autoclaved for one-half hour at 110° C. As checks on the sterility of the water, the surface of the gall, and the sand and mortar used in grinding the tumor, three plates were poured.

Toward noon (about 20 hours after treatment) the beet was uncovered, the thin dead surface now covering the tumor was removed with sterile scalpels and thrown into 10 c. c. bouillon. The volume of the gall was now measured in sterile water. It displaced 8 c. c. of water. A cube of the white flesh, approximately 4 by 4 by 4 mm., was cut out and thrown into 10 c. c. bouillon to duplicate Reinelt's experiment as nearly as possible (see later). The remainder was thrown into the mortar, cut into small fragments with sterile cold scalpels, several grams of the sand added, together with 40 c. c. of sterile water, and the material then ground vigorously for 15 minutes, i. e., until the beet was pulped and the fluid began to darken from oxidation. The mortar was tilted and the mass allowed to settle for 10 minutes, after which 20 c. c. of the fluid was recovered by means of sterile pipettes and put into sterile test tubes. All of this fluid was then distributed into 10 c. c. volumes of nutrient +15 agar, using a sterile pipette, and 68 Petri-dish plates were poured during the next three hours as follows: 5 received 1 drop each; 10 received 2 drops each; 5 received 3 drops each; 5 received 5 drops each; 5 received 10 drops each; and the remaining 38 received 0.5 c. c. each. The work was done in a clean culture chamber. The agar was inoculated and poured at 39° to 40° C. The three check plates gave the following results at the end of the seventh day:

No. 1, inoculated with 1 c. c. from a tube of 10 c. c. bouillon in which all the scrapings of the sterilized surface of the gall were allowed to soak for an hour—nothing.

No. 2, inoculated with 1 c. c. of washings from the dry heated white sand—nothing.

No. 3, inoculated with 1 c. c. of washings from the interior of the autoclaved mortar before using it—1 mold spore.

The 68 plates poured from the 20 c. c. fluid (contents of the tumor plus possible contaminations from the air) gave the following results:

(1) After 24 hours at 23° C.: Sixty-four plates sterile; 4 plates contain a total of 10 colonies.

(2) After 48 hours at 23° C.: Fifty-seven plates sterile; 11 plates contain a total of 17 colonies—2 of them a widespreading white organism. All the other colonies are tiny, white, and buried.

(3) After 72 hours at 23° C.: There are now 298 additional colonies all small, slow-growing, white, and buried. Twenty-seven plates are still sterile so far as the hand lens indicates, and a number of these received 0.5 c. c. volumes of the fluid. Plate 67 was rejected from the count because overrun and spoiled by a white colony.

(4) After five days at 23° C.: There are now 792 additional colonies not counting those on two plates (53 and 59) which are now overrun and spoiled by a white rapidly growing organism. All of these colonies are small, white, slow-growing, and most of them buried. Twenty-two plates are still free from colonies. These received the smaller inoculations, but much more fluid than one ordinarily expects to use, viz, most of them 2 to 10 drops.

(5) After seven days at 23° C.: (*a*) Thirty-seven plates show no additional colonies; (*b*) 2 more plates (50 and 57) rejected because contaminated; (*c*) on the remaining 29 plates there are 386 additional colonies, all small and mostly scattered, rarely small clusters on a tiny fragment of tissue.

Two plates of lot *c* contain each 1 Penicillium spore and 2 yellow ntruding colonies. The colonies which came up at the end of 24 and 48 hours may be regarded as contaminations from the air. Those on the 5 rejected plates may also be neglected. Of the remainder very few can be regarded as air borne.

(6) On the ninth day nearly all the white colonies are still buried and small and it is too early to say what proportion of these are the parasite. A few of the most hopeful-looking ones were transferred to bouillon.

(7) On the fifteenth day those previously transferred to bouillon as hopeful were rejected and 13 other colonies were marked for transfer, most of these having appeared after the ninth day—in other words, of the 1,500 colonies which developed only 1 per cent had the appearance on the plates of the right organism. Of these colonies only 2 proved pathogenic, i. e., produced tumors when inoculated into sugar beets, and these yielded very small slow-growing galls as if feebly virulent (Pl. XXXVI, fig. 1). It should be stated, however, that the beets were not growing much.

REINELT'S EXPERIMENT.

The experiment performed in Reinelt's manner gave the following result at the end of 5 days:

Second dilution from tube containing the cube—plate 4, nothing; plate 5, nothing; plate 6, 2 small white colonies, nature doubtful; plate 7, nothing.

The dilutions were made as follows: After the 4-millimeter cube, which was cut with a cold knife, had remained in the tube of bouillon about 10 minutes, one 3-millimeter loop of this fluid was transferred to tube 2, which was shaken; then two 3-millimeter loops from this tube were transferred to tube 3, which was shaken. The plates were then poured from tube 3, the following amounts of fluid being put into each: Plate 4 received three 3-millimeter loops; plate 5 received two 3-millimeter loops; plate 6 received one 3-millimeter loop; plate 7 received one 3-millimeter loop.

After the cube had stood in tube 1 for 2 hours it was mashed in the bouillon with a sterile scalpel as well as it could be (but not nearly as effectually as the remainder of the tumor which was ground with sand), and after standing an hour longer (to diffuse) 4 additional plates were poured, inoculating as follows directly from the tube containing the crushed beet: Plate 68 received three 3-millimeter loops; plate 69 received two 3-millimeter loops; plate 70 received one 3-millimeter loop; plate 71 received one 1-millimeter loop.

At the end of the fifth day all were free from gall colonies. The same was true at the end of 15 days.

OTHER ATTEMPTS AT ISOLATION.

At the same time as the above, Miss Brown made attempts to cultivate out the organism believed to be the cause of the sugar-beet tumor from galled sugar beets from Arlington, Va.; Blissfield, Mich.; and Fairfield, Wash. On her numerous poured plates she obtained a small sprinkling of colonies which appeared to be the right thing, and with subcultures from 30 of these made inoculations upon sugar beets in one of our houses, and also with the more hopeful a few inoculations upon daisies, tomatoes, etc. Of the whole lot inoculated, greatly to our surprise, not a single one has produced galls on sugar beet. The only results obtained up to the time this bulletin goes to the press are tiny beginnings of overgrowth in needle pricks on a few daisies and oleanders, and 2 somewhat larger, but small hyperplasias, on 2 tomato stems. There can be no doubt about the growths being tumor growths and due to what was inserted, but 5 colonies only of the 30 have yielded these results. The remainder have failed.

Remarks.—The explanation of the failures and of the very slow growth of the successful inoculations is a matter which must be left to the future. Two or three possible explanations may be offered. No great amount of energy was devoted to attempting to isolate the organism from sugar beets until after we had read Professor Jensen's paper late in the autumn of 1910. The gall on sugar beet then assumed a new importance in our eyes, and we made, as we have stated, diligent attempts to get out an organism with which the tumor on sugar beets could be reproduced. We began, however, not until the end of the growing season, namely, in November, when the galls were old, and although we plated from those which had no decayed spots on them, it is quite certain that the galls had nearly or quite approached the end of their growth for the current season, and may be assumed to have been several months old. We think, therefore, either that the right organism was dead for the most part in the tissues at this time, or so weakened by its own by-products or by reactions of the plant that it had lost its virulence. There seems to be no good reason, if one thinks about it, why an organism which loses its virulence in culture tubes might not also lose it in the interior of the host plant, if it had ceased or nearly ceased to stimulate growth, and had been subject for some weeks or months to harmful reactions resulting from its own products and those of the host itself. The fact that after 3 months of hard work, out of 42 colonies selected from several thousand as the most hopeful we have found only 7 able to produce tumors, shows how difficult it is sometimes to isolate a pathogenic organism from material known or believed to contain it.

HOP ON DAISY.

INOCULATIONS OF FEBRUARY 8, 1908 (BROWN).

Five plants of the Queen Alexandra variety of daisy were inoculated by needle pricks from a slant agar culture 5 days old. (For origin, see *Hop on Hop*, p. 90.) Four and 5 shoots were inoculated on each plant. The plants were old and growing slowly.

Result.—February 18, 1908: There were protuberances at all places of inoculation.

April 22, 1908: No large knots like those due to the daisy organism were produced, although there was definite evidence of infection in each plant.

INOCULATIONS OF FEBRUARY 10, 1908 (SMITH).

Twelve plants of the Paris daisy were inoculated with the hop organism from cultures of February 3. They were Nos. 1 to 12, inclusive, each made from a separate colony.

Result.—June 1, 1908: Nos. 1–7, 9, 11, and 12 gave no tumors. Plant 8, inoculated with colony 8 in 2 places, yielded a tumor about one-fourth inch in diameter. Plant 10, inoculated with colony 10 in 3 places, yielded a very slight tumor—a little round nodule about 2 mm. in diameter and 2 mm. in height.

Remarks.—This experiment may be interpreted in at least four ways: (1) That the plants were old and hard when inoculated and thus resistant; (2) that the organism had lost virulence in the gall; (3) that 10 of the 12 colonies were the wrong organism; (4) that the daisy had become somewhat resistant as the result of previous inoculations. Colony 8 is the only one that has given a tumor of any considerable size.

Inoculations of April 17, 1908 (Smith).

Four daisy plants, Nos. 1 to 4, inclusive, were inoculated with the hop organism from agar cultures 48 hours old.

Result.—June 1, 1908: No tumors.

Inoculations of April 25, 1908 (Brown).

Five more plants of the ordinary Paris daisy were inoculated with slant agar cultures of the hop organism 2 days old.

Result.—May 12, 1908: The same protuberances were formed as in the first set of inoculated daisies, but no well-developed galls.

Inoculations of May 9, 1910 (Brown).

Eight terminal shoots on 3 large plants ready to blossom and already inoculated on the lower part of the main stems with daisy gall and bearing galls received punctures introducing the hop organism from an agar culture several days old.

Result.—June 23, 1910: Seven floral shoots failed to take (the main stems below now bear large daisy galls). One vegetative shoot now bears at the punctured spot 2 small smooth galls each about as large as a pea and on directly opposite sides of the small branch. Six inches below the main stem bears a large daisy gall.

The reason for selecting daisy plants already bearing tumors produced by the daisy organism was that all the numerous uninoculated daisies of the same age were so much further advanced in flowering than these inoculated ones that no soft tissues were available.

Inoculations of November 12, 1910 (Smith).

The preceding inoculations of hop on daisy having given such slight results in comparison with hop on some other plants, daisies propagated from stock never before used were inoculated with subcultures 3 days old from agar colonies (fresh isolation, California, 1910).

Checks were held on sugar beet. Both daisies and beets, especially the former, were young plants in excellent condition for inoculation.

Result.—December 12, 1910: The subcultures from 2 of the colonies proved to be noninfectious. Colony 1 produced galls on each one of the 3 inoculated beets at the place of puncture. These tumors were, respectively, 1, 3, and 5 cm. in diameter at the end of 4 weeks, when the experiment was interrupted. The largest gall was on the most vigorous plant; the smallest was on a feeble plant. At this time 2 of the 4 daisies bore each a small gall on the pricked part. These galls were 2 to 3 mm. only in diameter. The other plants were free.

February 8, 1911: Three of the 4 daisy plants now bear tumors where inoculated and not elsewhere. The smallest one is 1 cm. in diameter, the largest one is 3 cm.

Inoculations of November 30, 1910 (Smith).

The preceding experiment was repeated on 12 young growing daisy plants, using subcultures of the hop organism (colony 1) and inoculating from peptone bouillon cultures 16 days old.

Result.—February 8, 1911: Eight plants remained free, 4 developed tumors at the place of inoculation and not elsewhere. These are now one-eighth to one-half inch in diameter.

Inoculations of December 2, 1910 (Smith).

A second repetition of the new hop (colony 1) on 20 daisy plants of the same character, using agar streak cultures 2 days old, gave the following:

Result.—February 8, 1911: Seventeen plants free from tumors. On 3 plants there are 4 tumors, the largest three-fourths inch in diameter, the others one-fourth inch.

HOP ON TOMATO.

Inoculations of November 21, 1908 (Brown).

Three tomato plants of a small, red, hothouse variety, 5 feet tall and in fruit, were inoculated with the hop organism. The stems about half way down the plant showed bulgings where roots might possibly protrude and adventitious roots also projected a distance of one-eighth to one-fourth inch. The bulging places on the stem and the smallest adventitious roots were both inoculated with agar streak cultures 3 days old. More than a dozen places on each plant were punctured. Two check plants were held, the punctures being made in the same way as those of the inoculations.

Result.—December 10, 1908: Galls formed at the bulged places on the plants inoculated, but no hairy-roots.

December 22, 1908: The hop-galls had increased so that they were now one-half to three-fourths inch in diamater. No galls formed on the adventitious tomato roots inoculated with the hop organism. The checks remained healthy.

February 15, 1909: Photographs were made (Pl. II, fig. 3).

HOP ON OLIVE.

INOCULATIONS OF MAY 9, 1910 (SMITH AND BROWN).

Ten rapidly growing olive shoots were inoculated near the tip by needle pricks from a young agar culture. For want of other material these inoculations were made on plants standing close together in a bed. Many of these bore olive tubercles as the result of recent inoculations, and other inoculations with the same organism (*Bacterium savastanoi*) were in progress at that time.

Result.—July 18, 1910: All negative except No. 4, which bears 25 needle pricks, from 3 of which small galls have developed. Possibly these are olive galls, as branches of the same plant were by accident inoculated two days later with the olive-tubercle organism and the gardener sprayed the plants every day.[a] Otherwise it is difficult to account for the 9 failures, as the shoots have grown very rapidly since inoculation, i. e., 16 inches to 2 feet, and were all inoculated with equal care and from the same culture.

December 7, 1910: Plates were poured in gelatin from one of these galls and only the olive-tubercle organism was isolated, thus confirming the previous supposition.

HOP ON COTTON.

INOCULATIONS OF JULY 20, 1910 (BROWN).

Inoculated 6 young growing cotton plants (Willet's Red Leaf) at the crown with 5-day-old cultures of the hop-gall organism. Two checks were held.

Result.—October 21, 1910: All negative; growing conditions good.

HOP ON GRAPE.

INOCULATIONS OF APRIL 16, 1909 (BROWN).

Eight young shoots of European grape on 3 plants, variety not known, were inoculated with agar streak cultures 2 days old. The crowns of the 3 plants were inoculated also. Two plants were held as checks, the stems and crowns being punctured with a sterile needle.

Result.—May 6, 1909: The stems of the inoculated vines had small knobbed prominences like the regular grape gall. No galls showed on the crown.

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[a] The strain of the olive-tubercle organism used proved very infectious, every one of the 208 inoculations yielding a gall with many subsequent metastases, although all of the 105 inoculated plants had previously borne galls, and two former strains (one from California, one from Italy) had ceased to be infectious to them. The recent strain was isolated from an olive knot sent by Miss Florence Hedges from Portofino, Italy, in April, 1910.—E. F. S.

HOP ON ALMOND.

INOCULATIONS OF APRIL 16, 1909 (BROWN).

Three almond trees grown from the seed, and which were over a year old, were inoculated on the stems and the crown with agar streak cultures 2 days old. No checks were held, as there were but 3 trees.

Result.—May 6, 1909: No indication of galls forming.

August 21, 1909: Galls formed on both crown and stems of 2 trees. The inoculations did not take on the third tree. This tree was smaller, gnarled, and not in a good growing condition.

November 19, 1909: A photograph was made (Pl. XV, fig. 2).

HOP ON PEONIA.

INOCULATIONS OF MAY 6, 1909 (SMITH AND BROWN).

Three roots of *Peonia officinalis* were inoculated with the hop gall organism. Checks were held.

Result.—September 2, 1909: The plants were knocked out of the pots and examined. No galls were found on those inoculated. We were led to make these inoculations because Dr. Whetzel reported root galls on peonia in New York.

HOP ON SUGAR BEET.

INOCULATIONS OF APRIL 17, 1908 (SMITH).

Six sugar beets were inoculated from agar subculture 48 hours old.

Result.—May 18, 1908: Tumors have appeared and are three-fourths inch or more in diameter (Pl. XII, fig. 1).

June 1, 1908: All of the inoculated sugar beets bear tumors. The largest tumors are now 2 inches in diameter.

INOCULATIONS OF MARCH 7, 1910 (BROWN).

Five sugar beets were inoculated on the crowns by needle pricks from 3-day-old agar cultures.

Result.—April 20, 1910: Galls are forming in the pricked parts.

May 11, 1910: All contracted the disease and developed tumors several inches in diameter (Pl. XXI). Other beets in the same and adjoining rows remained free from the disease.

Remarks.—The hop organism used for the last inoculations on sugar beet had been in the laboratory a long time, having been transferred (without passage through plants) to fresh slant agar and bouillon once a month for over 2 years, i. e., about 26 times, to keep it alive, and yet with all of these transfers it remained actively pathogenic.

(See also checks, Hop on Daisy, Nov. 12, p. 86.)

HOP ON HOP.

On October 23, 1907, a hop root from California with a good-sized gall was brought into the laboratory by Dr. W. W. Stockberger for examination. Plates were poured from this gall and the gall organism obtained.

INOCULATIONS OF NOVEMBER 21, 1907 (BROWN).

Only 4 hop plants could be obtained, and these were inoculated by needle pricks at the crown with cultures 2 days old.

Result.—January 15, 1908: The plants had made almost no growth, but 3 of them had small galls at the inoculated places.

Isolation of organisms.—On January 28, 1908, some more hop roots with irregular galls 4 to 6 inches in diameter were received from California through Doctor Stockberger. Parts of these galls were blackened and decayed; some swarmed with nematodes and some had small white nodules of new gall tissue on the margin of the old blackened gall tissue. These young portions were used for pouring agar plates, and 7 days later the gall colonies appeared on the plates.

INOCULATIONS OF JUNE 10, 1908 (BROWN).

Eight seedling hop plants grown in the greenhouse were inoculated at the crown with slant agar cultures 4 days old. Four other seedlings were punctured with a sterile needle for checks.

Result.—July 20, 1908: Galls 1 to 2 inches in diameter had formed on all the inoculated plants. The checks bore no galls.

CHESTNUT ON DAISY.

INOCULATIONS OF NOVEMBER 13, 1908 (BROWN).

Four shoots of a daisy plant were punctured and a little of a pure culture introduced on the point of the needle (same cultures used on sugar beets).

Result.—December 2, 1908: The inoculations showed only as a slight swelling.

December 19: Perceptible galls are now visible on the daisy at the points of inoculation.

March 13, 1909: The galls are now 1 to 1½ inches in diameter (Pl. XVI, fig. 1). They grew more slowly than any gall heretofore observed except perhaps peach on daisy (inoculations of February 3, 1908) and some of peach on apple (inoculations of March 11, 1908).

May 3, 1909: The galls are still growing, nearly 2 inches in diameter, and quite tough.

CHESTNUT ON GRAPE.

INOCULATIONS OF JUNE 10, 1910 (BROWN).

In 1910 a fresh strain of the chestnut gall organism was plated from an old gall growing on the crown of a young chestnut (material from Mr. David Fairchild). A single shoot of *Vitis vinifera* was inoculated by needle pricks from a subculture on agar.

Result.—June 24, 1910: Distinct small knobs are visible on some of the needle pricks. Altogether about a dozen are now visible.

October 31, 1910: Slight elevations in the needle pricks, but no true galls.

CHESTNUT ON SUGAR BEET.

On November 7, 1908, among a lot of chestnut trees received by the Department of Agriculture there was one with a gall 3 inches in diameter on the stem. This gall was rather soft and of a texture much like that of a nut meat. Plates were poured from it and the gall colonies appeared 4 days afterwards.

INOCULATIONS OF NOVEMBER 13, 1908 (BROWN).

Five young sugar beets were inoculated with colonies from the plates poured November 7, which colonies were 2 days old (visible 2 days). The beets were grown in an open bed in the greenhouse.

Result.—December 26, 1908: All the inoculated sugar beets had knots three-fourths to 1 inch in diameter (Pl. II, fig. 4). They were white and not so hard as the ordinary galls of daisy and peach.

January, 1909: The galls on the sugar beets rotted off before the end of the second month.

INOCULATIONS OF MAY 24, 1910 (BROWN).

Five medium-sized sugar beets were inoculated from an agar streak by needle pricks on the root.

Result.—July 18: All negative.

INOCULATIONS OF JUNE 10, 1910 (BROWN).

A fresh isolation called "new chestnut" was made out of a rather old gall procured from the estate of Mr. David Fairchild, in Maryland. Five beets were inoculated on the roots by needle pricks.

Result.—July 18, 1910: Negative.

POPLAR ON OLEANDER.

INOCULATIONS OF JULY 20, 1910 (SMITH).

Six young shoots on vigorous plants were inoculated by needle pricks from a peptone water culture 5 days old.

Result.—October 22, 1910: One shoot missing; the other 5 diseased, but only in the pricked area. The results in detail are as follows: (1) Four rounded knots (one-eighth to one-half inch in diameter); 16

pricks failed. (2) Four rounded tumors, largest one-half inch; several pricks failed. (3) Missing. (4) Three very small knots (one-sixteenth to one-eighth inch); rest of pricks failed. (5) Six small rough galls, each about one-eighth inch in diameter, 5 on stem, 1 on petiole; 10 pricks failed. (6) Six small rounded tumors (one-eighth to one-quarter inch).

POPLAR ON OPUNTIA.

INOCULATIONS OF JULY 5, 1910 (SMITH AND BROWN).

Growing branches were inoculated by needle pricks from an agar streak culture 4 days old. Four varieties were used, having the following appearance, viz: (*a*) Elongated, dark-green joints, which are uniformly fine-hairy, and nearly free from prickles, i. e., short, weak spines; (*b*) smooth, light-green, elliptical, or pear-shaped, thin joints, with inch-long, single spines; (*c*) like *b* but with clusters of spines; (*d*) somewhat like *b*, but with round flat branches, very long spines, and clusters of brownish short prickles.

Result.—October 21, 1910: (*a*) Negative, one shoot inoculated; (*b*) one of the two pricked joints has, where inoculated, a smooth round tumor half an inch in diameter; (*c*) two young shoots, negative; (*d*) two shoots, negative.

December 19, 1910: The tumor on *b* is smaller than it was, and nipple-like projections have appeared on it.

February 8, 1911: The tumor has not increased in size.

POPLAR ON COTTON.

INOCULATIONS OF JULY 20, 1910 (BROWN).

Inoculated the crowns of 6 young growing cotton plants (Willet's Red Leaf), with a 5-day-old 2 per cent peptone water culture of Flats poplar gall organism. (For origin see "*Poplar on sugar beet,*" p. 93.) Held 2 checks.

Result.—October 21, 1910: All negative. The plants grew well.

POPLAR ON GRAPE.

INOCULATIONS OF JUNE 4, 1910 (SMITH AND BROWN).

Two small shoots of *Vitis vinifera* were inoculated in the green terminal growing parts by needle pricks with an agar subculture from a colony derived by poured plate from a gall found on the trunk of a large tree of *Populus deltoides* (*P. monilifera*) in Washington (Flats below the Washington Monument).

Result.—July 18, 1910: One of the inoculated shoots bears a dozen small tumors, and the other 26 (Pl. XXIV, *C*). There are no galls on the plants except where they were pricked.

POPLAR ON APPLE.

INOCULATIONS OF JULY 20, 1910 (SMITH AND BROWN).

Six shoots were inoculated by needle pricks from an agar streak culture 5 days old.

Result.—All grew well. One yielded in the pricked spot a distinct, rounded, corky tumor one-fourth inch in diameter. The other 5 failed.

POPLAR ON BRASSICA.

INOCULATIONS OF JULY 20, 1910 (SMITH).

The organism derived from the Flats poplar was inoculated by needle pricks into growing stems, using a peptone water culture 5 days old: (1) Early Summer cabbage; (2) Early Wakefield cabbage; (3) collard; (4) Tall Green Scotch kale; (5) kohl-rabi.

Result.—October 21, 1910: (1) The four inoculated plants bear galls in the pricked area and not elsewhere. On one they are small; on the others they are 1 to 2 inches in diameter (Pl. XXXIII, fig. *B*). Eleven uninoculated plants in the same pots are free. (2) Two plants bear large galls (1 to 2 inches in diameter), but only in the pricked areas; 6 checks are free. (3) One large collard bears a dozen small knots (one-fourth to one-half inch in diameter) in pricked area and not elsewhere. One-half of one knot shows hairy-root (Pl. XXXIII, fig. *A*). No other collards. Cabbage checks free. (4) One plant has 2 well-defined small galls (one-fourth inch) in pricked area and none elsewhere. Eight checks free. (5) Top of inoculated plant missing, i. e., broken off by someone (this was the pricked part); 10 checks free. These stood close to the Wakefield cabbage and the kale.

POPLAR ON SUGAR BEET.

INOCULATIONS OF JUNE 4, 1910 (SMITH AND BROWN).

In May, 1910, an organism resembling *Bacterium tumefaciens* was plated from a gall on *Populus deltoides* (called "Flats poplar" because the tree stands on the Flats near the river below the Washington Monument in the District of Columbia). These galls grew in clusters on the extreme base of the trunk of a large tree, but were not large; i. e., only 1 to 3 inches in diameter.

The material used for inoculation was an agar streak subculture (May 31) from a poured-plate colony. Ten sugar beets in a row were inoculated on the root toward the crown by needle pricks.

Result.—July 5, 1910: 100 per cent of infections. Galls began to develop at once in the pricked parts, but the plants were allowed to

remain in the bed until this date that they might get larger. Each of the 10 plants now bears a good-sized tumor, and at least half are larger than the root which bears them (Pl. XXII, *B*, *C*). The surface is white or pinkish, and they have not yet begun to decay. Material for sections was fixed in Carnoy. No checks were held, but the adjoining row inoculated at about the same date with an isolation from a chestnut gall might be considered as a check row, since the numerous needle pricks have yielded nothing.

Remarks.—Cultures were also made from the large old poplar gall shown on Plate XXIII, and subcultures from 2 of the most hopeful looking colonies (called Newport No. 1 and Newport No. 2) were inoculated into sugar beets (upper part of root) by needle pricks on June 30, 1910, but without results. Nine plants were inoculated, but the weather was hot and they did not grow much. The examinations were made on July 18.

POPLAR ON CALLA.

Inoculations of July 5, 1910 (Brown).

The corms of 8 growing callas were inoculated with 4-day-old agar cultures of the Flats poplar organism. Both leaves and corms were in good condition.

Result.—October 25, 1910: Examined the callas and found a pebble-like outgrowth on 1 of those inoculated; also knobbed portions on 2 others. Plates were poured from 2 corms, but no gall colonies appeared.

WILLOW ON DAISY.

Inoculations of May 9, 1910 (Brown).

In the spring of 1910 a small willow gall was received from South Africa. Plates were made from it, and colonies obtained which resembled *Bacterium tumefaciens.* A subculture from one of these colonies was pricked into 3 terminal shoots of old, slow-growing daisies already bearing large daisy galls near the ground.

Result.—June 25, 1910: Two of the shoots bear smooth brown galls one-half inch or more in diameter at the place inoculated. The third shoot, which is yellow and sickly, has not developed any.

WILLOW ON WILLOW.

Inoculations of December 12, 1910 (Smith).

Six recently rooted small cuttings of *Salix babylonica* were inoculated on rather slow-growing young shoots by needle pricks, using a 4-day agar streak culture which was a subculture (possibly the twelfth) from a colony plated from the South African willow gall in December, 1909.

Result.—January 5, 1911: Typical small galls (Pl. XXXV, fig. 1) have appeared in a portion of the needle pricks on 4 of the 6 plants, 5 shoots, 14 galls in all.

RELATION OF SO-CALLED HARD-GALL OF APPLE TO SOFT-GALL.

BOTH KINDS OF CROWN-GALL DUE TO BACTERIA.

Doctor Hedgcock has distinguished between hard and soft gall of the apple. He has not pointed out any good means of separating the two, but has stated the more common hard-gall to be noninfectious. As a matter of fact, the two kinds of tumors under consideration intergrade and both are due to bacteria, the differences being referable probably either to variation in the rate of growth of the host plant or else to varying degrees of virulence on the part of the bacteria, perhaps to both of these factors.

APPLE-GALL (HARD AND SOFT) ON VARIOUS PLANTS.

PRELIMINARY ISOLATIONS AND INOCULATIONS OF 1908 (BROWN).

On October 15, 1908, an apple seedling with a gall 1¾ inches in diameter was found among trees purchased by the Department of Agriculture to be used for the congressional distribution. An organism very much like the daisy gall organism in appearance and manner of growth was plated from this gall. Inoculations into apple trees, peach trees, daisy plants, and sugar beets produced galls in each species, although the per cent of infections was low.

On November 25, 1908, apple-galls were received from a nursery in Maryland. Plates were poured from the softest of these knots and the same organism obtained as before.

On November 27, 1908, Dr. G. G. Hedgcock brought in some of his so-called hard-galls of apple and challenged us to plate out the gall organism from them. These, as well as those used for the previous work, were galls without the accompanying tufts of roots (hairy-root). Plates were poured. Colonies resembling the gall organism appeared on the plates, and inoculations into sugar beets proved that it was the gall-forming organism, for in 18 days galls were produced at the inoculated places. The plants were from the vicinity of Washington (second D. C. test).

On November 4, 1908, Doctor Hedgcock also sent hard-galls of apple from Iowa to Doctor Smith, asking that tests be made. Miss Brown plated out what seemed to be the gall organism from one of these plants, but no inoculations were made by her. For result of independent isolations and inoculations into daisy by Doctor Smith, see inoculations of November 9, 1908.

HARD GALL OF APPLE ON DAISY.

INOCULATIONS OF FEBRUARY 24, 1908 (SMITH).

Six Paris daisy plants were inoculated with 4-day-old slant agar cultures (from beef-bouillon culture of February 18, from stock agar stab of January 6) of Doctor Hedgcock's first (D. C.) apple gall. Each plant was inoculated in two places in the top.

Result.—June 1, 1908: No tumors. Plants discarded. Same cultures were negative on apple of January 23, 1908.

INOCULATIONS OF OCTOBER 22, 1908 (BROWN).

Four daisy plants were inoculated on the upper part of the stem from colonies on plates poured October 15. These colonies, however, had not appeared until October 20, so that the greater part of the cultures were in reality only 2 days old.

Result.—November 6, 1908: Small knotty growths had formed on all the daisies; not like the regular daisy gall in shape, but more like that of the hard gall of apple.

August 21, 1909: The galls have increased in size very materially, as shown by the photograph (Pl. XV, fig. 1).

INOCULATIONS OF NOVEMBER 9, 1908 (SMITH).

Eight young Paris daisy plants were inoculated from cultures plated November 4 out of a very hard gall of the apple, forwarded by Doctor Hedgcock from Iowa. A 1-millimeter loop was usually scraped across a half dozen of the small colonies in order to get enough material, and then this was rubbed on a small area on the surface of the stem near the top of the plants and pricked in with a sterile needle. What remained on the platinum loop was rubbed over the wounds afterwards. As checks, the daisy plants were punctured in another place with a sterile needle. For this purpose plants were selected which had twin branches, one branch being inoculated and the other check pricked. The gardener was directed to withhold water for a few days until the check wounds should have healed over, so that the organism might not be scattered from the surface of the inoculated part into the pricks on the other branch. The plate used for these inoculations was photographed (Pl. XXV, fig. *D*).

Isolation of organisms.—The details of making these poured plates, their later appearance, etc., are as follows:

Two plants only of the hard gall were sent. The galls were found to be very hard indeed and not much raised above the surface of the apple stem. The surface of one of the galls was washed and then soaked for 3 minutes in 1:1,000 mercuric-chloride water. It was then

dug into and plates poured (November 4). After 2 days they yielded scattering saprophytic colonies of two types: (1) Whitish, circular, rather dense, of moderately rapid growth; and (2) larger, dendritic white ones, consisting of a large nonmotile schizomycete. At this time there appeared to be nothing else on the plates, but 2 days later the plates came up thickly with small, round, white colonies, and on the fifth day these had grown sufficiently so that there appeared to be very little doubt of their being the same sort of organism that we had plated from the crown-gall of the peach. These surface colonies were mostly less than 1 mm. in diameter, wet-shining, very translucent, white, circular; the buried ones were elliptical.

On November 9 transfers were made to agar streaks from 8 of these small colonies. One of the characteristic plates was then selected for the above inoculations. Plates were now made from the second hard gall of the same lot, and these yielded similar colonies, with which galls were also produced.

Result.—November 16, 1908: As yet no indications of tumors on any of these plants.

July 22, 1909: Galls have appeared. The best growths are about one-half inch in diameter and raised above the surface of the stem one-fourth inch or less. They are typical hard galls. Only about half the plants contracted the disease. This appeared at the inoculated spots, and not elsewhere. The growths resembled the original hard gall from which they were taken, rather than the ordinary daisy gall (Pl. III, bottom, stem 21).

Inoculations of November 18, 1908 (Smith).

Three daisy plants were inoculated from colonies on poured plates made November 9 from Hedgcock's second Iowa gall. At the time of inoculation the small colonies were white, dense, fleshy, circular, wet-shining.

Result.—July 22, 1909: Galls on each one of the three plants (Pl. III, bottom, stems 64, 65, 66). The growths are about one-half inch in diameter, and raised above the surface of the stem one-fourth inch or less. They are typical hard galls, i. e., not like the soft, rapid-growing daisy galls.

HARD GALL OF APPLE ON TOMATO.

Inoculations of December 4, 1908 (Brown).

The protruding adventitious roots of some nearly full-grown tomato plants were inoculated with the apple gall organism obtained from plates poured November 27. The projections were well out from the stem as though the roots were going to take hold of the soil. The cultures used were agar slants 2 days old, the first subculture

from the poured-plate colonies. The stems of the tomatoes were not inoculated.

Result.—February 2, 1909: No appearance of galls or hairy roots.

HARD GALL OF APPLE ON PELARGONIUM.

Inoculations of November 9, 1908 (Smith).

Two growing shoots of Pelargonium were inoculated from poured-plate colonies made November 4 from the hard gall of apple received from Iowa. These inoculations were from the same plate as the daisies inoculated on this date and were made in the same manner (p. 96).

Result.—November 16, 1908: As yet no indications of tumors on any of the plants.

April, 1909: No tumors appeared.

Inoculations of June 24, 1910 (Smith).

Ten growing shoots of *Pelargonium zonale* (pink and red flowered varieties) were inoculated in the soft terminal portion by needle pricks from an agar streak culture 2 days old. This was descended from a colony isolated in 1908. Four similar shoots were pricked with a sterile needle as checks.

Result.—August 10, 1910: Negative.

October 21, 1910: Negative.

HARD GALL OF APPLE ON APPLE.

Inoculations of January 23, 1908 (Smith).

Inoculated 10 growing Wealthy apple trees, part of them on the stock, with a bacterium plated from a hard knot on an apple tree furnished by Doctor Hedgcock (first D. C.). The roots were washed thoroughly and 50 or 60 pricks on each tree were made in some peculiar form, so as to recognize the place of inoculation. The gray-white slime from 44-hour-old slant agar cultures grown at 30° C. (5 subcultures, all descended from one small, white colony) was used for these inoculations. These cultures came from three 10-day-old slants and those from stock cultures made from the colony. After inoculation the trees were planted in 10-inch pots in the greenhouse.

Result.—No infections. Trees continued to grow, but very slowly. The colony used was, perhaps, a wrong one. Many small round white colonies appeared on the set of plates from which this colony was selected.

INOCULATIONS OF FEBRUARY 24, 1908 (SMITH).

Three Wealthy apple trees were inoculated with 4-day-old slant agar cultures (from beef-bouillon culture of February 18, from stock agar stab of January 6) of Hedgcock's first (D. C.) apple gall. Four inoculations were made on each plant, 2 into young shoots and 2 into old stems.

Result.—June 1, 1908: No tumors. Same set of plates as the preceding.

INOCULATIONS OF OCTOBER 22, 1908 (BROWN).

Three apple trees were inoculated on the crown and on the stem from hard-gall colonies on plates poured October 15. The colonies, however, had not appeared until October 20, so that for the most part the cultures were in reality only 2 days old. The apple trees were small and in a poor condition.

Result.—November 24, 1908: Two of the 3 apple trees had small galls at the crown. One of these trees bore 2 galls, one at the crown and the other a little below the crown.

December 22, 1908 (see Pl. II, fig. 2).

INOCULATIONS OF NOVEMBER 12, 1908 (SMITH).

Four apple trees were inoculated from colonies on poured plates made November 4 from Doctor Hedgcock's first Iowa apple gall. When used for inoculation the colonies were white, dense, fleshy, circular, wet-shining. The trees were leafy but growing very slowly. They are of the same lot that failed to take daisy inoculation (p. 43), and earlier root inoculations (January 23, 1908) with organism from first (D. C.) hard gall of apple. Each of the 4 trees was inoculated on the root just underground; 2 of them on 2 roots each, and 2 also on parts aboveground—1 in 1 place and the other in 3 places.

Result.—Nothing. Trees growing very slowly. The same organism inoculated into daisies gave slow-growing hard galls.

HARD GALL OF APPLE ON SUGAR BEET.

INOCULATIONS OF NOVEMBER 12, 1908 (SMITH).

Nine sugar beets were inoculated from poured-plate colonies (each from a separate colony) made November 4 from first hard gall of apple received from Iowa. At the time of inoculation the colonies were white, dense, fleshy, circular, wet-shining.

Result.—Negative. Plants small and making scarcely any growth.

INOCULATIONS OF DECEMBER 4, 1908 (BROWN).

Eight young sugar beets were inoculated with subcultures 2 days old from apple-gall colonies. These were descended from the same poured-plate colonies (second D. C.) used for the tomato inoculations of this date (p. 97). The beets were in a cool house making a slow growth.

Result.—December 18, 1908: Galls formed on only 2 of the beets and these were not more than half an inch in diameter. The beets had not grown much since the time of inoculation.

INOCULATIONS OF JUNE 24, 1910 (BROWN).

This is the culture recorded under "Morphology" as "old apple" and now believed to be something other than the crown-gall organism. Ten beets were inoculated on the roots by needle pricks from a young culture.

Result.—July 18, 1910: All negative.

HARD GALL OF APPLE ON MONSTERA.

INOCULATIONS OF NOVEMBER 12, 1908 (SMITH).

Six root tips (aerial roots) of *Monstera deliciosa* were inoculated from colonies on plates poured November 4 from Hedgcock's hard gall of the apple (first Iowa). When used for inoculation these colonies were white, dense, fleshy, circular, wet-shining.

Result.—No galls. Some of the roots bifurcated owing to injury of the growing point.

RELATION OF CROWN-GALL TO HAIRY-ROOT.

HAIRY-ROOT OF APPLE DUE TO BACTERIA.

Originally we had no intention to touch the subject of hairy-root, but Doctor Hedgcock having expressed a belief that it was not due to any organism and having sent on material with the request that we examine it, plates were poured and inoculations were made with the following results:

On November 7, 1908, an apple tree with small roots in clusters on the main root was sent in from Iowa by Doctor Hedgcock to Doctor Smith to experiment with for isolation of the hypothetical organism which we believed to exist therein and he did not. There was no typical gall on either roots or stem, but there were small enlargements at the base of the little clusters of hairy roots. A few of the rootlets of the bunched mass were rather fleshy. The root-

lets themselves were not used, but the thickened bases were cut out and used for pouring agar plates. In 4 days the characteristic gall colonies appeared. In 5 days they were of good size and looked very much like the colonies obtained from apple galls. This was the first time any such organism had been isolated from hairy-root.

EXPERIMENTS TO DETERMINE WHERE THE ORGANISM IS LOCATED.

November 9, 1908.—In order to find out whether the organism believed by us to be the cause of the hairy-root of apple was located in the main root under the point of origin of the hairy roots, or in the fleshy small roots themselves, plates were poured from material cut from these two locations. Colonies came up on those plates which has been made from that portion of the main root lying under the base of the hairy-root tuft, but none at all on the other plates; i. e., the organism was not found in the hairy roots themselves.

November 27, 1908.—An apple tree affected with hairy-root was brought in from his Washington, D. C., plantation by Doctor Hedgcock, who challenged us to prove the presence of an organism. The clustered roots were not dry and wiry, but fleshy and tender. Where they joined the main root there was a broad, flat enlargement. Plates were poured from the fleshy roots and also from the enlargement at the base of these roots. As in the previous experiment, the gall colonies appeared only on the plates poured from the thickened base.

February 16, 1909.—Some apple trees affected with hairy-root were sent to Doctor Smith by Doctor Whetzel, plant pathologist in Cornell University, for us to prove the presence of a pathogenic organism. The roots were very dry and had to be soaked before they could be cut. Little knobs grew on the main root just where the clustered roots came out, and these were used as material with which to pour agar plates. In four days the typical gall colonies were up on the plates and were used to infect young apple trees.

HAIRY-ROOT ON DAISY.

INOCULATIONS OF MAY 9, 1910 (BROWN).

Six terminal shoots on old slow-growing daisy plants were inoculated with the hairy-root organism.

Result.—June 25, 1910: Four shoots are negative; 2 show several tiny galls growing out of the inoculation pricks. These do not bear any roots. The stems lower down bear large daisy galls.

HAIRY-ROOT ON TOMATO.

Inoculations of November 21, 1908 (Brown).

In connection with the inoculations of the apple-gall organism into the adventitious roots of tomato, the hairy-root organism was also tried on tomato. The tomato plants were of the same sort as those used for the apple-gall inoculations, viz, a small, red, hot-house variety, 5 feet tall and in fruit. The stems about halfway down the plant showed bulgings where roots might possibly protrude, and adventitious roots projected a distance of one-eighth to one-fourth inch. Both the bulging places on the stem and the smallest adventitious roots were inoculated from agar streak cultures 3 days old. Three plants were inoculated, more than a dozen places on each being punctured.

Two check plants were held, the punctures being made in the same way as those of the inoculations.

Result.—December 10, 1908: No trace of hairy roots or galls on the plants inoculated with the hairy-root organism.

December 22, 1908: No galls or hairy roots formed on the adventitious tomato roots inoculated with the apple hairy-root organism.

The checks remained healthy.

Inoculations of December 10, 1908 (Brown).

A second test of the apple hairy-root organism on tomato was made, much younger plants in a better growing condition being inoculated with 3-day-old cultures of the hairy-root organism. The nascent roots on the stem were treated in the same way as the first set.

Result.—January 2, 1909: No effect produced on the stem of the plant or on the nascent roots by the inoculation.

Inoculations of April 1, 1909 (Brown).

Ten young tomato plants about 6 inches tall were inoculated with agar streak cultures of the apple hairy-root organism 2 days old. The crown of the root, the middle of the stem, and the growing point of the stem were inoculated. Four checks were held.

Result.—May 2, 1909: Examined the plants and found that 6 had roots projecting in a cluster, but whether these were adventitious roots put out to hold the plant in position or due to the presence of the organism could not be determined. The checks had not these decided rootlets, but the inoculated plants did not always have the rootlets in the immediate area of the puncture. The plants were replaced in larger pots and left to grow.

September 3, 1909: Decided that no hairy roots had formed.

HAIRY-ROOT ON YOUNG APPLE TREES.

Inoculations of April 5, 1909 (Brown).

Twelve young apple trees entirely free from gall or hairy-root were washed carefully and 8 of them inoculated with 2-day-old agar cultures of the apple hairy-root organism (Whetzel tree). Twenty-five pricks in groups of 5 were given each root, beginning at the crown and going down. The stem was notched to indicate the side inoculated. Four checks were held, the punctures being made in the same way.

Result.—May 3, 1909: Turned back the soil, examined, and found that hairs were forming in the pricked places.

September 3, 1909: Dug the trees, washed, and examined them. Five of the 8 showed very good cases of hairy-root (Pl. XVIII, figs. 1 and 2). Two failed and one bore rather small hard galls without hairy-root. One of the checks also had several small galls. This tree must have become infected during the planting. Plates were made from one of the hard galls obtained from the inoculated tree which did not bear hairy roots and an organism isolated. This was successfully inoculated into sugar beets on November 11, 1909, *both galls and hairy roots* developing. This indicates that the apple gall was actually due to the hairy-root organism, as suspected.

HAIRY-ROOT ON QUINCE TREES.

Inoculations of May 21, 1909 (Brown).

Three quince trees were inoculated with a 2-day-old agar culture of the apple hairy-root organism. The trees were in the greenhouse and just starting to bud out. The wood was very tough, but the stems were inoculated at the nodes and internodes; at least 30 punctures were made on each stem.

Result.—September 3, 1909: Negative.

November 28, 1910: (*A*) Three galls bearing hairy-root (Pl. XXXIII, fig. *D*) on stem well aboveground; (*B*) one stem gall, no roots from it; (*C*) several small galls on stem, one bearing hairy roots.

Five checks on the same bench remained free; also 10 plants of same lot inoculated with the organism marked "Quince."

HAIRY-ROOT ON SUGAR BEET.

Inoculations of November 13, 1908 (Brown).

Six young sugar beets growing in an open bed in the greenhouse were inoculated just below the surface of the ground, care being taken not to puncture along the line of the root hairs. The inoculations were made with agar-plate colonies of the apple hairy-root organism 2 days old. One daisy plant was also inoculated.

Result.—December 2, 1908: Examined the beets and found that fine roots were growing out at the punctured places, and little warty growths were at the bases of these roots. One and sometimes 2 roots protruded from a punctured place. This was observed on 4 out of the 6 beets.

December 22, 1908: Hairy roots were found on 1 more of the inoculated beets, making 5 out of the 6. No galls like the distinct galls of the daisy, peach, or apple were produced.

The daisy did not develop either hairy root or galls, although it was under observation until April 17, 1909.

Inoculations of December 22, 1908 (Brown).

Eleven young sugar beets were inoculated with slant agar cultures of the hairy-root organism 3 days old, the second subculture from agar poured-plate colonies. The organism was obtained from the apple tree brought in by Doctor Hedgcock. The inoculations were made just below the surface of the soil.

Result.—January 9, 1909: Four of the beets were pulled up; clustered roots of rather a fleshy texture were found on all 4 at the place of inoculation. There was no possibility of confusing these roots with those that occur regularly on either side of the beet, for they were too near the crown of the beet and besides were growing from small nodules.

April 10, 1909: Three more beets were removed and examined. Each one bore typical hairy-root (clustered roots) at the point of inoculation, which was midway between the two lines of lateral roots (Pl. XVII, figs. 1 and 2).

April 29, 1909: The remaining four beets were removed and examined. Three of these showed undoubted hairy-root at the point of inoculation. The fourth one probably developed hairy-root, but was rejected from the count because the needle entered on the line of the lateral roots rather than on the smooth surface between them, the inoculation having been made when the plant was very small.

Inoculations of February 24, 1909 (Brown).

Seven small sugar beets were inoculated just below the surface of the ground with 1-day-old agar streak cultures of the apple hairy-root organism, the first subculture from poured-plate colonies obtained from one of the apple trees sent from New York by Doctor Whetzel.

Result.—March 10, 1909: Two beets were pulled up and examined; clustered roots were found on one of them at the inoculated places, which were on the smooth part of the beet.

March 31, 1909: Three more of the 7 beets had clusters of little roots at the inoculated places. The checks showed the punctured places healed; no hairs had formed.

INOCULATIONS OF NOVEMBER 11, 1909 (BROWN).

Eight sugar beets were inoculated with 6-day-old agar streak cultures made from colonies plated from gall on apple root produced by inoculation (p. 103) with culture of apple hairy-root organism derived from the Whetzel tree.

Result.—March 10, 1910: One beet was pulled and both galls and hairy-root found present.

April 7, 1910: The remaining 7 beets were pulled and typical hairy-root found on all of them. Three had both hairy-root and galls, i. e. clusters of roots (hairy-root) growing out of galls which were not large (Pl. XVII, fig. 3; Pl. XIX, figs. 1 and 2).

MISCELLANEOUS.

In 1910 organisms were isolated from salsify gall, turnip gall, and parsnip gall, and inoculated respectively into salsify, turnip, and parsnips, and each also into sugar beet, but all of the inoculations were negative.

DESCRIPTION OF BACTERIUM TUMEFACIENS [a] FROM DAISY.

MORPHOLOGICAL CHARACTERS.

Vegetative cells.—The daisy knot organism is a small schizomycete of variable length, but usually short and generally not over 0.6 to 1μ in diameter, unless treated with severe flagella stains. None as slender as 0.2 or 0.3 μ have been observed.

Taken directly from a gall and stained with gentian violet, the following measurements were obtained in 1909: Single rods, 0.6 to 1.0μ by 1.2 to 1.5μ; paired rods, 0.6 to 1.0μ by 2.4 to 2.8μ. These were obtained in the following way: The surface of a young gall was scraped, washed, and sterilized; thin slices were then placed in distilled sterile water on sterile slides and the bacteria allowed to diffuse out of the sections for an hour. The sections were then lifted with sterile forceps, the fluid dried and stained. Only scattering rods were visible.

When grown on agar for two days and stained with Loeffler's flagella stain (in 1907) its length was 2.5 to 3μ and its breadth 0.7 to 0.8μ, or occasionally a little wider. Some recorded as 6μ long were probably paired rods.

[a] Name first used in Science, n. s., vol. 25, April 26, 1907, p. 672.

Other slides made at various times and measured February 25, 1910, gave the following results:

(1) Van Ermengem: Average diameter 1.2μ; some less, a few more. Result on a second slide: Average diameter 1 to 1.2μ.

(2) Pitfield's (Smith), agar 24 hour: Average diameter 1.2μ, widest 1.75μ, narrowest 1μ. Occasionally Y-shaped rods. Pitfield's (Brown): Widest diameter 1.1μ; many less wide, i. e., 0.8 to 0.9μ.

(3) Carbol fuchsin, without a mordant (30 minutes) flagella visible: Average diameter 1.2μ.

(4) Löwit's stain: Average diameter 1.56μ.

(5) Loeffler's flagella stain (1909): Diameter 0.8 to 1μ; none seen wider than 1μ.

In making these measurements a Zeiss photomicrographic stand, 3-mm. apochromatic oil-immersion objective, No. 12 compensating ocular, and Schraubenmikrometer were used (Smith).

The rods are straight, have rounded ends, thin walls, and a uniform diameter.

When taken from young agar cultures, the limits of size are 1 to 3 by 0.4 to 1.8μ. Size of the majority 1.2 to 2.5 by 0.5 to 0.8μ. Occasionally one finds more than two rods attached, end to end, forming short chains (seldom more than 3 or 4 elements). Long chains have never been observed in the daisy organism except under abnormal conditions, e. g., in old 3.5 per cent salt bouillon, where sinous or curved rods 20 times the ordinary length were seen.

Endospores (?).—*No endospores have been observed, and probably none occur.* Certainly they are not formed under most culture conditions, as shown by the short life of cultures and by their sensitiveness to heat. The following additional experiments were made in 1910:

Bouillon cultures some weeks old were boiled for 3 minutes with the result that all were killed. The experiment was repeated after some months with the same result. Bouillon cultures were then heated for 20 minutes at 80° C., after which some grew. It was thought that owing to the lumpy character of the slime the heat might not have penetrated to the center of all the pseudozooglœæ. The experiment was repeated, therefore, exposing the tube for 60 minutes at 80° C. After this none of the transfers grew, not even when large quantities of the fluid were used (1 drop, 2 drops, etc.).

Flagella.—*The organism is motile by means of a polar flagellum.* Sometimes 2 or 3 terminal flagella are present, but more often in the slides examined there was only 1. The rods of young cultures show a distinct movement when examined in hanging drops. The flagella were first stained by the senior writer from 24-hour agar cultures, using Pitfield's flagella stain (fig. 1, *a*). Afterwards they were

stained by Miss Brown, using Loeffler's flagella stain (fig. 1, *c*), and subsequently Van Ermengem's stain (fig. 1, *b*). They were also stained by Miss Lucia McCulloch without a special mordant, by simply exposing the flamed covers to carbol fuchsin for from 30 to 60 minutes and then washing in alcohol (fig. 1, *d*).

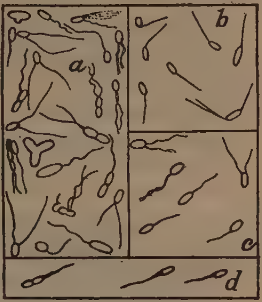

FIG. 1.—Flagella of *Bacterium tumefaciens* from daisy: *a*, Pitfield's flagella stain; *b*, Van Ermengem's stain; *c*, Loeffler's flagella stain; *d*, from a slide stained 30 minutes in carbol fuchsin without mordant.

Capsules (?).—The organism is viscid after some days on agar, etc., but capsules have not been demonstrated. Welch's stain was tried.

Zooglœæ (?).—Pseudozooglœæ occur, and perhaps the stringy masses in peptonized beef bouillon should be regarded as transitions toward zooglœæ. Under the microscope these masses consist of short rods held together by a viscid slime.

Involution forms.—Numerous involution forms (fig. 2) were observed in bouillon cultures which were making a slow growth at 0° C. The cultures were first examined under the microscope on the fourteenth day. Occasional Y-shaped rods occur in young agar cultures (fig. 1, *a*). Club-shaped and Y-shaped involution forms were also seen in salt bouillon and in bouillon and agar to which acetic acid was added. See also note on ordinary bouillon.

FIG. 2.—Involution forms of daisy organism after two weeks in bouillon at 0° C. Bottom growth: *a*, drawn by E. F. S.; *b*, drawn by Brown. Similar involutions forms were produced in young agar cultures and also in bouillon by exposure to acetic acid.

BEHAVIOR TOWARD STAINS.

This organism when taken from young agar cultures stains readily in all ordinary basic anilin stains so far as tried, e. g., gentian violet, fuchsin, carbol fuchsin, amyl Gram, methyl violet. It is not surrounded by any substance that interferes with staining. When stained from a 2-day agar streak in Loeffler's alkaline methylene blue, the rods were either a uniform pale blue or showed round to oval, inner portions bearing a much heavier stain. There were sometimes two of these bodies in a rod, but more often one and that usually polar. About one-fourth of the rods stained in this manner, and the part not heavily stained was of a uniform pale blue.

It does not stain by Gram. It stains readily a uniform deep blue if amyl alcohol be substituted for ethyl alcohol in the washing after exposure to the anilin gentian violet and the iodine-potassium iodid of Gram's stain.

Taken from beef bouillon 7 weeks old it did not show glycogen stain when exposed to iodine water; i. e., there was only a uniform yellow color.

It is not acid-fast.

Brizi's method was employed on daisy galls without success. By the use of methylene green (not methyl green, but that was tried also), without subsequent exposure to acid, numerous cell inclusions, consisting of bacteria-like granules, were demonstrated, but whether really bacteria remained undetermined.

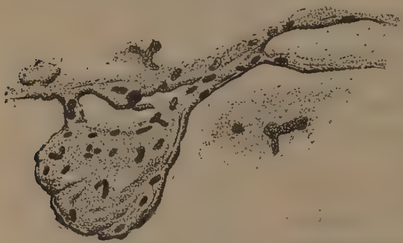

FIG. 3.—Daisy organism. Slime from pellicle on beef-bouillon culture three weeks old. Stained with carbol fuchsin, and camera-drawn by Miss Brown.

The bacteria Brizi succeeded in staining readily in poplar tumor tissues by an acid-fast method were probably not this organism. In our hands the gall-producing organism in American poplar galls stains like the daisy. It is not an acid-fast organism.

In sections it is often stained with difficulty, and we have seldom been able to differentiate it well from the surrounding tissue. It seems to us to occur, for the most part, at least, in the interior of the parenchyma cells rather than in the intercellular spaces or vessels. Repeated efforts to stain in situ have not yielded, as a rule, well-stained, sharply defined rods such as one would expect, but occasionally stained and unstained we have seen rods inside the cells which we believe to be the bacteria.

CULTURAL CHARACTERS.

NUTRIENT AGAR.[a]

Colonies.—When the organisms are obtained from a crushed knot the colonies come up in from 3 to 12 days (usually 4 to 6) at a room temperature of about 25° C. on poured agar plates. They come up very much slower when taken from knots than when taken from young cultures. The white smooth surface colonies are circular with an even margin, rounded up to the center, and have a shining semitransparent luster. The colonies increase in size slowly at 25° C.,

[a] +15 peptonized beef bouillon with 1 per cent Merck's agar flour.

attaining their maximum size of 2 to 4 mm. in thin-sown plates in from 2 to 4 days after becoming visible. They attain a larger size on culture media after many transfers than when first plated from the galls. The colonies, especially when they pile up in the center, are opaque, but never of a chalky white. They often resemble the watery colonies of some forms of the root-tubercle organism of legumes. Plates poured from a young bouillon culture show colonies sometimes in 24 hours, and nearly always in 48 hours at 25° C. Old colonies are sometimes iridescent.

Streaks.—Needle stroke not wide-spreading and very translucent at first. On slant agar (+15) the streak made with a platinum needle has a moderate filiform growth and does not branch on the surface nor penetrate the agar. The white streak widens slowly (chiefly toward the bottom), is slightly to considerably raised, sometimes nearly convex in cross section, and has a glistening luster; it is opaque or translucent and is slightly opalescent; is free from odor, somewhat slimy (especially after the first two or three days), and does not color the agar upon which it grows—at least not for some weeks, after which it may sometimes show a slight brownish color. In one strain (1909) the old slime, especially where it had run down into the V, had a trace of brownish in it lighter than Ridgway's tawny olive and somewhat resembling his buff or cream buff. This culture looked suspicious, but proved pathogenic. This has happened a number of times. The rods taken from this substratum stain readily with carbol fuchsin, gentian violet, methylene blue, or anilin methyl violet.

Two streaks from "B," made February 14, 1907, on slant 6 per cent glycerine agar by use of a loop, covered at the end of 48 hours the whole surface of the agar with a smooth, wet-shining growth, which was white by transmitted light. This growth was slightly viscid and plainly alkaline. There was no stain of the agar, nor were there any crystals. The cultures were pathogenic, as shown by inoculations of February 18 on daisy, tomato, and tobacco (Smith).

Stab.—Nontypical in stab cultures. The growth is filiform and best toward the top of the stab; the surface growth is scanty to abundant and generally restricted. Agar is not liquefied nor softened.

CORN-MEAL AGAR.

Feeble growth at end of five days. This experiment was repeated twice with the same result—growth at the end of four weeks was very slight, the media being made by the same formula, but in another laboratory.

POTATO.

On sterile potato cylinders (lower end in water) the organism makes a much more rapid growth than on agar. The growth is first visible

along the line of the streak, which is slightly elevated with entire margin. It spreads rapidly and in from one to two days covers the entire surface of the cylinder. The white growth has a smooth surface with a wet-glistening appearance. It has a slimy to viscid consistency, is free from odor, and turns the potato cylinder a grayish color, which becomes darker with age. It is never yellow on potato. The organism has but little action on the potato starch and its growth on potato is correspondingly transient.

STARCH JELLY.[a]

Growth scanty; diastasic action absent or feeble; medium unstained or only slightly stained. Some years later the experiment was repeated and continued for a longer time with the same result, except that the old daisy strain, which had become noninfectious, now stained the medium brown.

NUTRIENT GELATIN.[b]

Colonies.—Colonies dense, white, circular, small, nonliquefying. In plates poured March 26 from a 3-day-old bouillon culture carried to a second dilution the colonies were numerous, but remained very small. The surface colonies were 1 to 1.5 mm. at the end of 4 days at 20° C. and were not larger 2 days later (Brown). In thinly sown plates the growth of the surface colonies was slow, the largest being 2 mm. in diameter at the end of 24 days at 16° C.; they were round, white, dense, flat to raised, with entire edge, and no sign of liquefaction (Smith). They were never yellow with fringed margins.

Streaks.—There is a very *good growth, starting off slowly* at a temperature of 11° C. on gelatin streak cultures.

This experiment was repeated two years later, using gelatin from another laboratory with identical results. The gelatin was +12 on Fuller's scale. The temperature varied from 9° to 10° C. There was very slight growth up to the end of the third day. At the end of 12 days there was a good white growth.

At room temperatures (22° to 23° C.) on the same gelatin at the end of 2 days the streaks were as good as streaks of the same age on +15 peptonized beef agar, but this parallel growth did not continue. At the end of 4 days the agar streaks showed a copious growth, while the gelatin streaks showed only a moderate growth. At the end of 13 days the gelatin streaks were pure white, wet-shining, smooth on the surface, with bunches (tufts) of small crystals projecting from the under surface of the streaks into the unstained gelatin. Some white slime had also run down into the V. There was no liquefaction.

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[a] For composition, see "Bacteria in Relation to Plant Diseases," vol. 1.

[b] Peptonized beef bouillon with 10 per cent Nelson's photographic gelatin No. 1 and made +10 on Fuller's scale with sodium hydroxid.

Stabs.—In gelatin stabs the growth is best at the top; the line of puncture is filiform; liquefaction is absent, and the medium is not stained. It did not make an abundant growth.

LOEFFLER'S BLOOD SERUM.

Medium not liquefied. After 14 days at about 26° C. the growth along the streak was moderate, filiform, flat, glistening, and smooth; color white, tending toward cream in the old cultures. The medium was slightly grayed below the condensation water.

NUTRIENT BEEF BROTH.[a]

Clouding often absent or inconspicuous; a rim of gelatinous threads and more or less pellicle; also in young cultures very delicate suspended short filaments, best seen on shaking. In 48 hours after inoculating from bouillon there was no surface growth and no clouding; there was a slight, usually filamentous sediment, which became visible upon shaking the tube containing the culture. On thorough shaking the fluid becomes thinly clouded with numerous white suspended delicate threads (1 to 10 mm. or more in length). Under the microscope these threads are seen to be made of innumerable closely compacted small rods several times as long as broad. In 4 days a ring had formed, but the clouding of the liquid was either absent or not noticeable, although long gelatinous threads extended from the ring at the top to the bottom of the broth. These threads appeared to flatten out at the lower ends, forming a flocculent sediment. In cultures 3 weeks old the same phenomena were conspicuous. When stained with carbol fuchsin, there were many much swollen, irregularly staining, vacuolate, branched slime threads containing bacteria (fig. 3). In old cultures (7 weeks) the strings when examined under the microscope appeared in the form of irregular fine threads more or less vacuolate. At first these threads were taken for bacterial filaments undergoing disorganization, but on further study and careful staining they proved to be slime threads containing numerous involution forms and unmodified bacteria. No odor is noticeable in these cultures. There is often no true pellicle, only what might be termed an interrupted one. At other times, especially on standing for some weeks undisturbed, a true pellicle forms which fragments on shaking. It may be mentioned, however, that in a strain under cultivation for three years a continuous thick firm (nonfragmenting) pellicle finally formed, and coincident with this the virulence greatly lessened.

Sometimes in 12 hours at 25° C., when inoculated copiously from a young culture, the bouillon contains *numerous suspended delicate filaments* easily visible, especially on shaking.

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[a] Containing 1 per cent Witte's peptone and sodium hydrate to read +15 on Fuller's scale.

ALKALINE BEEF BROTHS.

The organism grows better in acid than in alkaline bouillons. The optimum reaction in peptonized beef bouillon lies between +12 and +24 of Fuller's scale. In May, 1908, growth was found to be more rapid in +15 bouillon than in phenolphthalein neutral bouillon. This was true at end of the second and the eighth day. The inoculations were from an agar culture 4 days old.

This experiment was repeated in 1910 with +15 bouillon, neutral bouillon, and −15 bouillon. At the end of two days there was most growth in the +15 and least in the −15. All showed more or less clouding, especially the alkaline ones. At the end of five days at 25° C. there was a plain white rim in the +15 tubes, and a very scanty one in the others; doubtfully present in some of the −15 tubes. On shaking, the fluid was at least three times as cloudy in the +15 bouillon as in the 0 or −15. The tubes were inoculated from 10 c. c. of sterile water in which a loop from a 2-day agar culture was diffused by shaking. For tests in other grades of alkalinity see following table and the chart under Comparative Tests.

SUGARED PEPTONE WATER.

Heavy pellicle, long continued growth, and final brown stain of the fluid, in flasks of autoclaved river water containing Merck's c. p. dextrose, Witte's peptone and c. p. calcium carbonate. Frequently the pellicle settled and a second one formed. The cultures were alive at the end of 4 months when turned over to the chemist for examination.

MILK.

Coagulation delayed; extrusion of whey begins only after several days; coagulum not peptonized (?). There is usually a pellicle or interrupted pellicle. For example: Four tubes of sterile milk were inoculated with a 1-millimeter loop of beef-broth culture 3 days old and kept at 23° C. Two days after inoculation no change had taken place in the consistency of the milk. Six days after inoculation the only change noticeable was the formation of a very shallow layer of whey on the surface of the inoculated milk. This is the customary behavior in milk. Often separation of whey is long delayed.

In 6-months-old milk cultures, three-fourths dried out, but still alive, the color of the gelatinous curd was Rhamnin brown No. 2 nearly (Repert. de Couleurs, Soc. Fr. des Chrysanth.), or between drab and ocher of Standard Dictionary (spectrum). Under the microscope, the bacteria were in the form of short rods, single and end to end in pairs, mostly as a pure white precipitate, 3 mm. wide, which

did not take stain very readily (carbol fuchsin). No tyrosin crystals were found (hand lens and compound microscope), and if there is any solution of the curd it is very slow and incomplete.

At the end of 10 days, tubes of milk inoculated with the daisy organism in February, 1910, showed about 2 mm. depth of whey on top of a separating curd, which remained fluid. At the end of the twenty-fourth day there was a small amount of clear whey over a copious fluid curd, beneath which was a small amount of clear white bacterial precipitate. Color much as in checks, which were brownish from overheating.

On May 9, 1910, 6 additional inoculations were made from 3 of the 6-months-old milk cultures into sterile white milk (i. e., milk not overheated), 3 check tubes being held. At the end of 9 days the only visible change was a white pellicle on the inoculated milks. Examined July 1, the inoculated tubes contained about 1 centimeter depth of clear whey supporting a well-defined white pellicle and resting on a homogeneous-looking opaque white curd about 3 centimeters deep (Pl. XXV, figs. *g*, *h*). The curd was not browned and yet not as white as in the checks. This so-called curd was fluid, as shown by gentle shaking.

There is never any rapid separation and digestion of the curd such as Brizi describes for his *Bacillus populi*.

LITMUS MILK.

The litmus is gradually blued, then reduced. Inoculations into litmus milk, using a 1-mm. loop of a 3-day-old beef-broth culture, resulted in 8 days in a deeper blue color (indigo blue); and in 24 days the blue color disappeared with a slight formation of whey at the surface. It is apparent from this test and others which were subsequently instituted (Table XII) that the culture is alkaline from the start, the litmus becoming reduced later. The litmus is never reddened. The behavior in litmus milk indicates the presence of a lab ferment. The reduction of the litmus may be partial or complete and is always slow. There was not much, if any, peptonization of the curd, and the whey at the end of 2 months was dark by reflected light (not red), the curd being either bluish, drab, or wholly bleached.

SILICATE JELLY.

Slow white growth. (See p. 156.)

COHN'S SOLUTION.

Growth scanty or absent, medium nonfluorescent. Many tests were made.

USCHINSKY'S SOLUTION.

Growth scanty, not viscid. Tubes were inoculated from a beef-broth culture 3 days old, a 1-mm. loop being used for each tube of Uschinsky's solution. In 2 days a scanty growth, which was slightly flocculent, could be seen, together with a few white filamentous flaky particles which were in suspension in the liquid. At the end of 6 days no further change was perceptible and the fluid did not become viscid, nor fluorescent. There was no pellicle. Under the microscope, at the end of 2 months, the filamentous flakes consisted of numerous short rods staining readily in carbol fuchsin. These rods appear to lie in an unstained slime. No chains were detected.

SODIUM CHLORIDE BOUILLON.

Four per cent of salt inhibits growth, 3 per cent retards growth or inhibits it.

A 1-mm. loop of a 3-day-old +15 peptonized beef-broth culture was placed in each tube of peptonized beef broth, containing 1, 2, 3, 4, 5, and 6 per cent c. p. sodium chloride—several tubes of each sort. At the end of 6 days, growth was apparent in tubes containing 1, 2, and 3 per cent, but no growth could be detected in tubes containing 4 per cent or more of sodium chloride, indicating that 4 per cent will inhibit the growth of the organism. The growth in the 3 per cent was slight.

In another experiment the daisy organism refused to grow in 3 per cent salt bouillon (Table VI).

In a repetition test it grew in 3.5 per cent.

GROWTH IN BOUILLON OVER CHLOROFORM.

Growth is unrestrained. Chloroform to the amount of 5 c. c. was run into 5 tubes of bouillon by means of a sterile pipette. Three tubes were then inoculated with the organism from a 10-day-old bouillon culture. In 2 days there was a good growth at the top of the bouillon; 12 days after inoculating a heavy growth was present. The tubes were not shaken.

NITROGEN NUTRITION.

Nitrogen is obtained from peptone, asparagin, etc. In filtered river water containing 0.5 per cent dextrose and 0.5 per cent urea there was no growth. The experiment was repeated some months later with the same result In filtered river water containing 1 per cent asparagin the organism made a slow initial growth, first visible after 5 days. Strain B, which had been in the laboratory several years and had lost its virulence, grew better than a recent isolation. This

indicates ability of the organism to take both its nitrogen and its carbon from asparagin. A decidedly less amount, but some growth, was obtained in river water containing only dextrose. In river water alone no growth was obtained. For further data see tables under *Observed Differences in Organisms from Various Sources.*

BEST MEDIA FOR LONG-CONTINUED GROWTH.

Milk, bouillon, dextrose peptone water with calcium carbonate are the best media we have tried. In tubes of milk the organism has lived for six months.

QUICK TESTS FOR DIFFERENTIAL PURPOSES.

The following are recommended tests:

Gelatin and agar plates, especially time of appearance of colonies on +15 agar plates made from the tumors; young agar stroke cultures; behavior in milk and litmus milk; growth on potato; behavior in Cohn's solution; behavior in the thermostat at 37° C.; stringy ring and suspended filaments in peptonized beef bouillon; inoculations into young, rapidly growing daisy shoots or into growing sugar-beet roots.

FERMENTATION TUBES.

No gas is produced, and the organism is aerobic in its tendencies. A basal solution was made by adding 2 per cent of Witte's peptone to water. Six solutions were then made from this, each containing 1 per cent of the following carbon compound: Glycerin, cane sugar, mannit, dextrose, maltose, lactose. One-half dozen fermentation tubes were filled with each of these solutions and sterilized by heating 20 minutes on three days in succession. Four tubes of each set were inoculated and 2 were left for control. The inoculated tubes each received a 1 mm. loop from a 2-day-old culture growing in water containing 2 per cent Witte's peptone and 1 per cent glycerin. Four days after inoculation there was a slight cloudiness in the open end of all inoculated tubes. The clouding was most conspicuous in the tubes containing dextrose and this extended down to the elbow. Next to the dextrose in point of cloudiness stood the maltose with threadlike thickenings floating at the surface. At the end of 10 days the cloudiness in the dextrose tubes had extended slightly into the closed end. A distinct deposit had also formed and particles of solid matter were floating in the liquid in the clouded part of the tube. Maltose had clouded to the middle of the U; the mannit solutions were clouded slightly beyond the U into the closed end of the tube, and a distinct deposit had formed. The cane-sugar and milk-sugar solutions were clouded almost to the bend in the tube, and

the clouded part was distinctly separated from the clear part as if by a veil. The glycerin solution contained a fine white cloudiness different in appearance from any of the other fluids.[a] At the end of 14 days the clouding had extended up the closed end of the tube, about 1½ cm., in the solution containing dextrose and that containing cane sugar. Eighteen days after inoculation the tubes were tested with neutral litmus paper. In the tubes containing glycerin, mannit, and lactose the paper turned blue and in the dextrose and cane-sugar solutions it turned slightly red. In the tubes containing maltose there was no change in the sensitized paper.[b] No gas formed in any of the tubes, nor were any of them clouded throughout the whole of the closed end. All the control tubes remained sterile.

AMMONIA PRODUCTION.

Moderate to strong.

NITRATES.

Nitrates are not reduced. Five tubes each containing 10 c. c. peptonized beef broth to which had been added just enough nitrate of potash to make 1 per cent nitrate-bouillon solution were inoculated with the daisy organism. At the end of four days the tubes were distinctly clouded and tests were made for nitrites as follows: To 10 c. c. of the nitrate bouillon containing the growing organism 1 c. c. of boiled starch water and 1 c. c. of potassium-iodid solution (1:200) were added. A few drops of strong sulphuric-acid water (2:1) were then added, but no trace of a blue color resulted, indicating that no nitrites had formed. This experiment was repeated several times at long intervals with subcultures from various isolations, but always with the same result. (See p. 148.)

INDOL.

Indol is produced in small quantity and very slowly. In 1908 several tubes of Uschinsky's solution with 1 per cent Witte's peptone added were inoculated with fresh agar cultures of the daisy organism. The inoculated tubes showed marked growth in four days and a test was made for indol, using concentrated sulphuric acid, and dilute sodium nitrite (1:200 in water). This test showed no trace of indol, even upon heating to 80° C. after the sulphuric acid and nitrite were added. This test was repeated at the end of 10 days, but again with negative

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[a] We were not able to duplicate this in subsequent cultures and now think it may have been due to precipitation of some of the peptone on standing.

[b] This experiment was repeated two years later with practically the same result—the neutral litmus paper showing only the barest trace of alkalinity after the cultures were 16 days old. After three months the fluid was clear, or nearly so, until shaken. There was enough white precipitate to make the unstained fluid flocculent filamentous turbid on shaking. It was still neutral to litmus paper.

results. This experiment was twice repeated with the same negative results.

In 1910 another trial was made inoculating into river water containing 2 per cent Witte's peptone and testing after 26 days' growth. This time the results were positive. There was a trace of pink before heating, and after 5 minutes in the water bath at 80° C. there was a decided red about half as deep as that given by those strains of *Bacillus coli* which are considered to be typical indol producers.

The indol reaction can not be obtained at the end of 24 hours, and seldom sooner than the eighth to tenth day.

TOLERATION OF ACIDS.

Slight toleration for citric, malic, and acetic acids. For the first tests 0.5, 1, and 2 per cent of the first two acids were added to tubes of neutral bouillon. A 7-day bouillon culture was used for inoculating the acid media. In six days there was some cloudiness which was least in the tubes containing 2 per cent acid. This cloudiness had not increased in any case a month after inoculating.

This test was repeated some years later with 1 per cent citric and malic acid, with negative results.

Tests were then made in 0.5 per cent citric and also in 0.5 per cent malic acid bouillon (+71) with negative results.

A final test was made in a beef bouillon containing 0.25 per cent citric acid (+34), and in another containing 0.25 per cent malic acid (+38). In the citrated bouillon of this strength both the old and the new strains of the daisy organism grew. In the malated bouillon only the old strain of the daisy organism grew (but there was only one test—1 tube). Undoubtedly the clouding observed in the first experiments (1907) should be attributed to chemical precipitates which in such acid solutions are frequently thrown out upon standing and become confusing.

One additional test was made in July, 1910, into peptonized beef bouillon acidulated to +26 with malic acid. Four tubes were inoculated. On the seventh day there was a bacterial pellicle on each one. The fluid was nearly clear—i. e., there was no fine clouding, but it contained strings, filaments, and flocks. The organism used was the newest strain of daisy.

The tests with acetic acid were made in 1911 adding it to both agar and bouillon cultures. Small amounts sterilized the cultures.

TOLERATION OF SODIUM HYDROXID.

The toleration for alkali is slight. Transfers were made to −15, −30, and −45 peptonized beef bouillon from a +15 bouillon culture 3 days old. Sixteen days after inoculating there was a slight growth

in the -15 tubes only. This experiment was several times repeated, with the same results.

Afterwards the organism was tested in -16 peptonized beef bouillon and in -34, with the following results: In the -16 the old strain (now nonvirulent) made a copious growth, and a more recently isolated virulent strain made a slight growth (about one-twentieth as much as the preceding). In the -34 the recent isolation made no growth, and the old strain (B) about one-twentieth as much growth as it did in the -16.

OPTIMUM REACTION FOR GROWTH IN BOUILLON.

The optimum reaction appears to lie between $+12$ and $+24$ on Fuller's scale. The first tests were made in May, 1908. Subsequent experiments (1910) gave the confirmatory results detailed in Table I, from which it appears that the organism is able to overcome moderate alkalinity and grow vigorously down to 0 on Fuller's scale. Judging from these results, the optimum (sodium hydroxid) alkalinity for growth in peptonized beef bouillon lies between $+12$ and $+24$ on Fuller's scale, and the limits for growth between -16 and some undetermined point between $+24$ and $+34$, the tubes being inoculated soon after the final titrations but left exposed at room temperature to the CO_2 of the air.

TABLE I.—*Relative growth* [a] *of daisy bacterium (newest strain) in peptonized bouillons of varying grades of alkalinity or acidity.*

[Inoculated from (A) 2-day-old peptone bouillon culture; also, for comparison from (B) water suspension of a 4-day agar streak, and from (C) subsequent young cultures. The +34 was acidulated with malic acid, the +36 with citric.]

Titration (grade of alkalinity or acidity).	(A) 2-day peptone bouillon culture.						(B) Water suspension of 4-day agar streak.					(C) Subsequent young cultures.			
	Three days.			Six weeks.			Three days.		Six weeks.			(1 to 2 months.)			
	Tube.[a] 1	Tube.[a] 2	Remarks.	Tube.[a] 1	Tube.[a] 2	Remarks.	Tube.[a]	Remarks.	Tube.[a] 1	Tube.[a] 2	Remarks.	Tube.[a] 1	Tube.[a] 2	Tube.[a] 3	Remarks.
−29	0	0		0	0		0		0			0	0	0	
−25	0	0		0	(b)		0		0			0	0		
−23												0	0	0	
−20	0	0		0	0		0		(b)			0	0		
−17												(?)	(?)	(?)	No growth or merest trace on bottom; no rim or pellicle; fluid clear.
−16	1	1		3	3	Suspended filaments; no pellicle.	1		3		Suspended filaments; no pellicle.				
0	2	2	No rim or pellicle; clouding with threads.	5	5	Crystals.	2	No rim or pellicle.	5		Crystals.				
+2	2	2	do.	5	5	Few large crystals.	2		5		Few large crystals.				
+7	2	2	Scant, fallen rim.	5	5	Fluid nearly clear; irregular crystals.	2	Scant, fallen rim.	5		Fluid nearly clear; crystals.				
+12	3	3	Fallen rim; better developed.	5	5	Coarse prismatic irregular crystals; fluid clear.	3	Fallen rim.	5		Crystals; fluid clear.				
+16	3	3	do.	5	5	do.	3	do.	5		do.				
+21	3	3	do.	5	5	do.	3	do.	5		do.				
+24	3	3	do.	4	4	Wide rim.	3	do.	4	4	Wide rim.				
+34												0			11 days only.
+36												0			

[a] Explanation of figures indicating growth: 0=no growth; 1=trace; 2=slight; 3=moderate; 4=good; 5=heavy, including pellicle.
[b] Broken.

VITALITY ON CULTURE MEDIA.

The life of this organism on culture media is brief to moderate. Often in hot summer weather agar streak cultures were found dead after 15 to 25 days when exposed to room temperatures. In cooler weather agar stab and streak cultures have lived for 4 or 5 weeks, but many observations made in the course of the prolonged inoculation experiments indicate that the organism is rather short lived on agar. It lives somewhat longer on agar kept in the ice box. Cultures freshly made from the galls have to be transferred as often as every three weeks if one would be certain of keeping them alive. The length of life of this organism is considerably prolonged by growing it in liquid media, notably in milk, in which it will retain its vitality for more than twice the length of time that it will on agar, whether kept in the ice box or at room temperature. (See Milk, p. 112.)

Flask cultures made in February, 1910, in river water containing 1 per cent dextrose, 1 per cent Witte's peptone, and some grams of calcium carbonate, were alive at the end of 7 months.

TEMPERATURE RELATIONS.

THERMAL DEATH POINT.

The death temperature is about 51° C., exposing for 10 minutes in +15 peptonized beef-bouillon. After several preliminary tests, e. g., at 43° to 53° C., it was concluded that the thermal death point must lie between these two temperatures. The following tests were then carried through in order to determine the point more accurately. Three sets of 4 tubes each of peptonized beef broth were inoculated with the daisy organism from 3-day-old slant agar cultures, and these tubes were placed in water at constant temperatures of 50°, 51°, and 52° C. At the end of 10 minutes the tubes were removed and kept for several days at about 29° C. At the end of 4 days growth appeared in all the tubes that had been exposed to 50° C. for 10 minutes. Slight growth appeared in some of the tubes that had been kept at 51 C., but no growth appeared even after 10 days in the tubes that had been kept at 52° C. for 10 minutes. This experiment was repeated several times with the same result, indicating that under the conditions named 51° C. is near the thermal death point of this organism.

OPTIMUM TEMPERATURE.

This appears to lie between 25° and 28° C. Growth at 25° C. on standard agar and in peptonized beef bouillon was three times better than at 30° C., and decidedly better than at 12° C. At the latter temperature growth was better in the bouillon.

MAXIMUM TEMPERATURE.

The highest temperature at which growth will take place is ±37° C. Several slant agar cultures were made (some from a 3-day-old agar culture, and others from a 9-day-old culture) and placed in a constant temperature oven at 39° C. No growth occurred. At the end of 3 days at this temperature some of the cultures were removed and kept at room temperature for 3 days, but no growth appeared. The control tubes gave a good growth. This experiment was repeated at 39° C. with the same result: No growth for 5 days, and none after removal to room temperature (5 days more).

Bouillon tubes were then inoculated and placed in a thermostat at 40° C., the controls being kept at room temperature. The controls grew. The tubes in the thermostat remained clear (6 days). Plates were then poured from them with negative results.

In another experiment glycerine agar streak cultures failed to grow at 37° C., but check tubes grew readily at room temperatures. The indications from these experiments are that a temperature of 39° to 40° C. soon destroys the life of this organism—under the conditions named. The following experiments were also made:

In March, 1910, in a well-regulated thermostat, carefully controlled, some very precise results were obtained confirming and extending the earlier observations. The temperature during the first 4 days ranged from 37° to 37.2° C. During the next 4 days the temperature increased a trifle, ranging from 37.2° to 37.5° C. This thermometer was compared with a standard instrument calibrated at the Reichs Anstalt in Berlin.

The experiment was begun at 9.45 a. m. March 1, by inoculating four +14 peptonized beef agar slants and 6 tubes of +15 peptonized beef bouillon from a peptone beef bouillon culture of February 26: One 3-mm. loop of the fluid was used for each agar slant and two 3-mm. loops for each tube of bouillon. One of the inoculated tubes of bouillon was kept at room temperature as a check and the other tubes were placed in the thermostat. At the end of 24 hours the tube at room temperature showed a moderate amount of growth. At the end of 48 hours there was a good growth in the check tube, but none in any of the 9 tubes exposed in the thermostat. At noon of March 4 there was still no growth in the thermostat. The same was true on March 7. On March 4, at noon, 1 tube of beef broth and 1 of agar were removed from the thermostat and put at room temperature. On March 5 at 3 p. m. another tube of beef broth and 1 of agar were removed from the thermostat. On March 7 at 11 a. m. the remaining tubes (3 beef bouillon, 2 agar) were removed from the thermostat.

On March 7 there was no visible growth in any of the tubes which had been in the thermostat. On March 9 the tube of bouillon removed March 4 showed numerous white bacterial flocks but no clouding. On March 14 typical strings appeared in the tube of bouillon removed March 4, and later on a pellicle. No growth took place in any of the other tubes.

Conclusion.—No growth in +15 bouillon or agar at 37° to 37.5° C. Exposure for 3 days retards subsequent growth at room temperature, and exposure for 4 days kills.

This experiment was repeated in the same thermostat using litmus milk, potato, slant peptonized beef agar (+16), and peptonized beef bouillon (+15). It was begun March 7 at noon. The range of temperature during the next 7 days was 37° to 37.4° C. Ten tubes (4 milk, 4 potato, 1 agar, 1 bouillon) were held as checks at 19° to 22° C. Thirteen tubes (5 milk, 4 potato, 2 agar, and 2 bouillon) were placed in the thermostat. All the checks showed distinct growth at the end of 24 to 48 hours. At the end of 4 days there was no visible growth in any of the tubes in the thermostat. On March 12 the 4 tubes of potato showed a trace of growth out of the water, i. e., at the extreme top of each cylinder. The 5 tubes of litmus milk were also now bluer than an uninoculated tube.

On March 14 (end of 7 days), the litmus milk was bluer than on March 12. There was still no visible growth either on the agar or in the bouillon, and that on the potato cylinders was scanty and restricted to the top. All the tubes were now removed to room temperature and agar streaks were made from the litmus milk. Two days later the agars streaked from the milk bore a good typical growth. Growth in the litmus milk and on the potato increased at room temperatures during the next week but no growth developed in the bouillon or on the agar.

Conclusion.—37° C. is above the limit for growth in +15 bouillon and on agar, and close to the limit for milk and potato. Exposure on agar or in bouillon for 7 days at 37° to 37.4° C. (mostly 37.1° to 37.3° C.) destroyed the organism.

MINIMUM TEMPERATURE.

Growth occurs at 0° C. Tests were made at temperatures varying from +10° to 0° C. with the result that growth was obtained even at the lowest temperature in peptonized beef broth and on agar. The ice box was used for temperatures above 3° C., and the records, made night and morning, were continued for a period of 2 weeks, or less if growth appeared earlier.

For tests of growth at 0° C. the experiment was continued for 2 weeks in the following manner: Transfers were made from 3-day-old agar cultures to agar and bouillon (2 tubes of each) which were cooled

to 0° C. before inoculating. The tubes were thrust far down into finely pulverized ice in a wine cooler which was set into the ice box close to large cakes of ice. Ice was added to the cooler night and morning after siphoning off the accumulated water. The temperature held constantly at 0° to 0.2° C. It was never above +0.2° C. The experiment was begun March 3, 1908. On March 7 there was a decided growth in the bouillon tubes but none on the agar. On March 9 a very slight growth was detected on the agar. On March 17, when the experiment was discontinued, the growth in the bouillon, although not heavy, was sufficient to show the usual characteristics. It was all at the bottom of the tube, none on the surface. There was enough growth on the agar to be visible, but it was slight. The bacteria in the bouillon were examined microscopically and many involution forms were found and drawings were made (fig. 2). Plates were poured from the bouillon and the daisy organism obtained in pure culture.

In order to obtain a still lower temperature, salt was mixed with the ice in a quinine can and the can was placed in a galvanized-iron bucket 10 inches in diameter. The bottom of the can, as well as of the bucket, was perforated to allow the water to escape. Both can and bucket were iced twice daily, using 4 tablespoonfuls of salt at each icing. The tubes were inoculated as before, and placed in the ice-salt mixture in which the thermometer was also placed. The bucket containing the ice and the quinine can with its contents was placed in the ice compartment of the ice box. Within a few minutes after the tubes were placed in the ice-salt mixture the contents had solidified and remained solid during the 2 weeks that the experiment was continued. The temperature varied from 0° to −14° C., but was never higher than 0° C. during the 2 weeks. At the expiration of this time the cultures were removed to room temperature where the beef broth quickly melted and was found to contain a distinct characteristic stringy growth, but only very slight. No growth was visible on the slant agar tubes.

EFFECT OF DRYING.

The daisy organism is killed readily by drying. An experiment made in April, 1910 (temperature 25° C.), gave the following result: Tiny drops of a bouillon culture 5 days old were spread on 25 clean, sterile, small cover glasses and set away on a shelf in the culture room (in diffused north light) in a covered, sterile Petri dish. The covers were then taken up by means of sterile forceps and dropped into tubes of sterile bouillon, one into each tube, with the following results:

Alive: Number of days dried, 1, 3, 7, 12.
Dead: Number of days dried, 2, 5, 6, 8, 9, 10, 13, 14, 15, 16.

The remaining 11 covers were dropped into 6 tubes of bouillon at the end of 20 days but no growth ensued (28 days). The character of growth of the daisy organism in beef bouillon renders it difficult to get an even, thin distribution and proper drying on cover slips, and to this is probably attributable the fact that the organism was alive on 3 of the 25 covers after the first day.

This experiment was repeated in June, 1910 (temperature 30° C.), using a peptone bouillon culture 5 days old and thinner smears, the covers being kept in the dark, with the following result:

Test begun at end of 2 days—
- (1) Two days, 2 tubes—no growth.
- (2) Three days, 1 tube—no growth.
- (3) Seven days, 16 tubes—no growth.

The 16 tubes were under observation for 13 days.

A second repetition in July, 1910 (temperature 30° C.), using a 6-day-old peptone bouillon culture, the covers being kept in the dark, gave the following results:

Test begun first day—
- (1) One day, 2 tubes—both grew, one very slowly.
- (2) Two days, 1 tube—no growth.
- (3) Three days, 1 tube—no growth.
- (4) Five days, 1 tube—no growth.
- (5) Six days, 1 tube—no growth.
- (6) Nine days, 1 tube—no growth.
- (7) Ten days, 1 tube—no growth.
- (8) Twelve days, 18 tubes—no growth.

The last lot was under observation for 10 days.

EFFECT OF SUNLIGHT.

Organism moderately sensitive to sunlight.—Agar tubes of the daisy organism inoculated from 3-day bouillon cultures were poured into Petri dishes and placed in the bright sunlight for 45 and 60 minutes, half of each plate being covered with black paper. After 4 days on each of the four plates there were numerous colonies under the covered parts, but none on the exposed parts. On 8 plates exposed at this time for shorter periods (30, 15, 10, and 5 minutes) colonies appeared on the exposed parts, but they were fewer than those on the shaded parts. Results similar to those just detailed were obtained by a repetition at 30 minutes. Colonies came up slower on the exposed side of the plate, but finally there were many. A second repetition gave similar results at 30 and 35 minutes. In a repetition at 35, 40, and 45 minutes, made a few weeks later (experiment begun April 3 in bright sunlight and final examination made April 11) tiny colonies appeared the third day on the shaded side of all the plates, but none at all developed on the exposed side (8 days).

ACIDS.

Acetic acid is produced in peptone water in the presence of grape sugar, and calcium carbonate (see report by Doctor Alsberg). Cane sugar is also broken up with production of an acid.

ALKALIES.

The blueing of litmus milk is due to ammonia. (See under *Crystals* [below]).

ALCOHOLS.

Ethyl alcohol is produced in peptone water in the presence of dextrose and calcium carbonate.

FERMENTS.

Invertase and lab are inferred to be produced: The former because an acid is produced and an invert sugar appears when the bacterium is grown in the presence of cane sugar; the latter because the casein is thrown down without the formation of an acid. (See under *Litmus Milk*, p. 154.) Litmus is also reduced.

CRYSTALS.

Prismatic crystals are formed in old cultures on agar partially neutralized by sodium hydrate (+15 agar), in bouillon and also in +10 nutrient gelatin. The washed crystals from bouillon cultures were determined for us by Dr. Carl L. Alsberg to be ammonium magnesium phosphate.

EFFECT OF GERMICIDES.

Copper sulphate.—This organism, as shown by poured-plate cultures, grew after 10, 15, and 20 minute exposures to 1:1,000 commercial copper sulphate in water, which had been acidulated with 19 drops of glacial acetic acid per 1,000 c. c. It did not grow after exposure for 30 and 40 minutes.

This experiment was repeated using copper sulphate 1:5,000, acidulated with 5 drops of glacial acetic acid per liter. Plates were poured after exposure of the organism to this solution for 10, 20, 30, 40, and 60 minutes. At the end of 6 days colonies appeared only on the plates made from the 10-minute exposure. This experiment was repeated a week later with the same result, namely, colonies only on the check plate and on the 10-minute exposure.

Exposures were made for 1 and 2 hours in 1:10,000 copper sulphate water with 8 drops of acetic acid per liter. At the end of 4 days there were numerous colonies on the check plate but none on the others.

Five days later this experiment was repeated, exposing 1¼ and 2 hours. At the end of 2 days there were colonies only on the control plate. Two days later colonies appeared also on the 1¼-hour plate, but none on the 2-hour exposure. Daisy plants sprayed with this strength of solution were not injured.

Formalin.—In September, 1907, tests were made in formalin diluted with water (1:500), exposing 10, 20, 30, 40, and 60 minutes. Colonies were abundant in all the plates except the 60-minute exposure, which yielded only a few.

Suspecting the strength of the formalin used, this experiment was repeated in May, 1910, as follows:

Transferred two 3-mm. loops of a 2-day-old bouillon culture to 10 c. c. of formalin in distilled water (1:500). This formalin solution was made from a freshly opened stock bottle. A check was made by transferring one loop from the bouillon tube to 10 c. c. of sterile water and pouring 2 plates.

The plates of the organism exposed to the formalin were poured at intervals of 10, 20, 30, 40, and 60 minutes.

Results: May 26, the plates are free from colonies. May 27, the plates are free from colonies. May 28, the check plates have numerous tiny colonies; the others are free. May 31, a few colonies up on the 10-minute plates; the others are free. June 4, colonies appeared only on the plates made from the 10-minute exposure.

Mercuric chloride.—Tests were made in a water solution of mercuric chloride (1:10,000), exposing 15 minutes, 30 minutes, and 5 hours, with a check plate of the organism from a suspension in distilled water. After 3 days there were numerous colonies on the check plate but none on the others and none appeared later.

PATHOGENICITY.

This organism was first isolated from galls occurring on the hothouse daisy (*Chrysanthemum frutescens*), but it causes, at least by inoculation, tumors in plants of many families, viz, Compositae, Solanaceae, Oleaceae, Umbelliferae, Vitaceae, Leguminosae, Rosaceae, Cruciferae, Caryophyllaceae, Chenopodiaceae, Urticaceae, Juglandaceae, Salicaceae.

LOSS OF VIRULENCE.

In cultures carried on for several years a slow gradual loss of virulence has been observed, but this was not detected until after the second year.

GROUP NUMBER.

The group number according to the descriptive chart, Society of American Bacteriologists, is: 212.2322023.

IMMUNITY.

Our studies are still incomplete. As far as they have gone they seem to indicate that repeated inoculations produce a heightened resistance to further inoculations with organisms of the original or of a lessened grade of virulence, but that more virulent strains will still produce galls on such plants, although the initial growth is usually slow.

OBSERVED DIFFERENCES IN CROWN-GALL ORGANISMS FROM VARIOUS SOURCES.

MORPHOLOGY AND BEHAVIOR TOWARD STAINS.

METHODS OF STUDY.

The measurements were made from 2-day agar streaks inoculated from agar streaks. The slime was diluted in sterile water and spread thinly on clean covers. These covers were then dried, flamed slightly, and stained with gentian violet. They were washed in water only, mounted in balsam, and examined at once. All the measurements were made by the same person (the senior writer) and represent the range of variation observed. All the rods were straight or nearly so, with rounded ends. With one or two exceptions all stained freely and uniformly. The measurements were made in the summer of 1910, using a Zeiss 2-mm. n. a. 1.3 oil-immersion lens and an eyepiece micrometer in a No. 6 compensating ocular, with a No. 12 compensating ocular for orientation and confirmation, using north light. One space of the Zeiss stage micrometer (1 mm. in 100 Th.) exactly equaled 12 spaces on the eyepiece micrometer, making one space on the latter equal to 0.833μ (confirmation of a determination by Miss Brown), but in making the measurements the value was for convenience reckoned at 0.8μ. The morphology did not vary greatly from culture to culture, as may be seen from the measurements given.

Similar young agar cultures were used for the demonstration of flagella (Pitfield's stain in most cases). The flagella were stained by Miss Katherine Bryan, but the slides were also examined by the senior writer.

For the acid-fast (Erlich Weigert) stain and the Gram's stain somewhat older agar cultures were used.

Old cultures were examined for spore formation, i. e., agar streak 19 days. For the examinations unstained in sterile water the top of the streak was used. It was then stained in carbol fuchsin 3 minutes and reexamined. Transfers were then made to sterile peptone water (3 mm. loop) from each tube and these were then at once

heated for 1 hour at 80° to 86° C., after which there was no growth (tubes under observation 23 days).

The name denotes the kind of gall from which the organism was isolated.

NEWEST DAISY.

A culture recently isolated and still infectious.

(*a*) Size 0.6 to 0.8 by 1.2 to 2.0μ. Most, I think, about 1.5μ long. Many short chains (4 to 8 segments) occur. Staining irregular and the protoplasm so pulled apart that it is very difficult to decide on the extreme length. Occasional club-shaped rods occur.

(*b*) Repetition two days later (unflamed): Size 0.5 to 0.7 by 1.0 to 3.0μ. Stained uniformly. The rods are single, paired, or in short chains often with indistinct constrictions, making it difficult to determine the maximum length of rods. The longest with indistinct septation are 4 to 10μ. A few are twice as broad as the multitude; a few are branched; a few are club shaped.

Acid fast.—Negative. Agar 6 days.
Gram's stain.—Negative. Agar streak 4 days.
Flagella.—Polar, 1 to 3. Pitfield's stain.
Spores.—Negative.

OLD DAISY (B).

A strain not now infectious: Size 0.4 to 0.6 by 1.0 to 2.4μ. Looks like the right thing. The bacteria adhere on the cover in small clumps. They are often paired and occasionally 4 are joined end to end. There is also an occasional club-shaped rod.

Acid fast.—Negative. Agar 5 days.
Gram's stain.—Negative. Agar 5 days.
Flagella.—Polar, 1 to 3. Pitfield's stain, etc.
Spores.—Negative.

PEACH.

Size 0.5 by 1.0 to 2.0μ.

Acid fast.—Negative. Agar 5 days.
Gram's stain.—Negative. Agar 5 days.
Flagella.—Polar, 1 to 3. Pitfield's stain.
Spores.—Negative.

HOP.

Size 0.4 to 0.8 by 1.0 to 1.6μ. Occasional club-shaped rods are present, but they are much less numerous than in the grape.

Acid fast.—Negative. Agar 6 days.
Gram's stain.—Negative. Agar 5 days.
Flagella.—Polar, 1 to 3. Pitfield's stain.
Spores.—Negative.

NEW ROSE.

Size 0.5 to 0.6 by 1.0 to 2.5μ, rarely 3μ.

Acid fast.—Negative. Agar 5 days.
Gram's stain.—Negative. Agar 5 days.
Flagella.—Polar, 1 to 2. Pitfield's stain.
Spores.—Negative.

OLD ROSE.

No measurements.

Acid fast.—Negative. Agar 5 days.
Gram's stain.—Negative. Agar 5 days.
Flagella.—Polar, 1 to 3. Pitfield's stain.
Spores.—Negative.

OLD APPLE.

Size 0.6 to 0.8 by 0.6 to 0.8μ. Often 3 to 8 elements in a chain. Almost like a streptococcus. Does not look like the right thing. Probably an intruder which has displaced the original pathogenic organism. Recent inoculation tests on sugar beet (June, 1910) gave negative results. (See also *Cultural Characters.*)

Acid fast.—Negative. Agar 5 days.
Gram's stain.—Positive. Agar 5 days.
Flagella.—Unable to stain.
Spores.—Negative.

APPLE HAIRY-ROOT.

Size 0.4 to 0.7 by 1.0 to 2.0μ. Occasionally one thicker.

Acid fast.—Negative. Agar 7 days.
Gram's stain.—Negative. Agar 5 days.
Flagella.—Polar, mostly 1 flagellum (Loeffler's stain). No results with Pitfield's stain.
Spores.—Negative.

NEW APPLE.

Descended from apple hairy-root, i. e., from apple gall which bore no roots but which produced both galls and hairy roots on sugar beet: Size 0.4 to 0.8 by 1.0 to 1.6μ. Occasionally one may be longer.

Acid fast.—Negative. Agar 6 days.
Gram's stain.—Negative. Agar 4 days.
Flagella.—Unable to stain.
Spores.—Negative.

ALFALFA.

Size 0.4 to 0.7 by 0.8 to 2.0μ. Average length about 1.2μ. Most are 0.5 to 0.6μ in diameter. In those which are 1.8 or 0.2μ long a slight equatorial constriction is usually visible. Occasional club-shaped rods are present.

Acid fast.—Negative. Agar 6 days.
Gram's stain.—Negative. Agar 5 days.
Flagella.—Polar, 1 to 2. Pitfield's stain.
Spores.—Negative.

GRAPE.

Size 0.5 to 0.9 by 1.0 to 1.5μ. Swollen and club-shaped rods 1.2 to 1.3μ in diameter are frequent. Perhaps degeneration forms.[a]

Acid fast.—Negative. Agar 5 days.
Gram's stain.—Negative. Agar 4 days.
Flagella.—Polar, 1 to 2. Van Ermengem's stain. No result with Pitfield's stain or Loeffler's stain.
Spores.—Negative.

NEW CHESTNUT.

Size 0.5 to 0.7 by 0.8 to 1.4μ. Seems to be rather shorter than most. The average length is about 1.0 to 1.2μ long. An exceptionally long pair measures 2.8μ, another average pair 2.2μ. The rods are single, in pairs, 4's or 8's, with distinct rounded constrictions. Pathogenicity not proved. Beets and grape inoculated gave no conclusive results.

Acid fast.—Negative. Agar 6 days.
Gram's stain.—Negative. Agar 5 days.
Flagella.—Unable to stain.
Spores.—Negative.

ARBUTUS UNEDO.

Size 0.5 to 0.9 by 1.2 to 2.2μ.

Acid fast.—Negative. Agar 5 days and bouillon 14 days.
Gram's stain.—Negative. Agar 5 days.
Flagella.—Unable to stain.
Spores.—Negative.

COTTON.

Size 0.4 to 0.6 by 1.0 to 2.4μ. Rarely as long as 2.4μ and most about 0.5μ in diameter. A few are club-shaped; a few are broader, and deeper stained than the majority. Slide overwashed.

Acid fast.—Negative. Agar 6 days.
Gram's stain.—Very feeble stain—should be estimated as negative. Agar 4 days.
Flagella.—Polar, 1 to 3. Pitfield's stain.
Spores.—Negative.

QUINCE.

Size 0.5 to 0.7 by 1.2 to 2.0μ. Greater tendency to short chains than in most of the slides so far examined, i. e., like newest daisy, but constrictions plainer.

Acid fast.—Negative. Agar 7 days.
Gram's stain.—Negative, i.. e., very feebly stained. Agar 1 day. Positive (same strain), agar 5 days. No satisfactory inoculations.
Flagella.—Polar, mostly 3. Stained by Loeffler's stain. No results with Pitfield's stain.
Spores.—Negative.

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[a] After 2 weeks on agar slant, Y's and swollen rods were very common, i. e., much more so than in any of the other 24 examined.

SUGAR BEET.

Organism which shows slightly pinkish on agar after a few days and is not infectious: Size 0.5 to 0.9 by 1.2 to 2.5μ. Two well-developed rods not yet separated measure together 0.8 by 3.2μ. Rods 2.5μ long without any distinct constriction are frequent.

Acid fast.—Negative. Agar 6 days.
Gram's stain.—Negative (feeble stain).
Flagella.—Unable to stain.
Spores.—Negative.

WILLOW FROM SOUTH AFRICA.

Size 0.4 to 0.7 by 1.2 to 2.4μ. Most of the rods are 1.5 to 2.0μ long. They are single or in pairs, occasionally in 4's joined end to end. The pairs frequently are curved a little. There are occasional club-shaped rods.

Acid fast.—Negative. Agar 5 days.
Gram's stain.—Negative. Agar 3 days.
Flagella.—Unable to stain.
Spores.—Negative.

POPLAR (FLATS).

Size 0.3 to 0.6 by 1.0 to 1.8μ. Most are 0.4 to 0.5μ in diameter. No spores are present.

Acid fast.—Negative. Agar 6 days.
Gram's stain.—Negative (a feeble stain).
Flagella.—Polar, 1 to 2. Pitfield's stain.
Spores.—Negative.

POPLAR (NEWPORT, R. I.).

Two colonies, which looked alike, were transferred from the poured plate, but they are not alike in morphology.

Colony 1.—Size 0.7 to 1.0 by 1.0 to 1.2μ. A very short, plump rod, with rounded ends, almost a coccus form, mostly paired. Not yet proved up and doubtful if the right organism. It has been inoculated into sugar beet with negative results.

Acid fast.—Negative. Agar 5 days.
Gram's stain.—Negative. Agar 5 days.
Flagella.—Unable to stain.
Spores.—Negative.

Colony 2.—Size 0.4 to 0.5 by 0.8 to 2.0μ. Mostly 1.0 to 1.4μ long. The rods are longer than those of colony 1. Sugar beet inoculated unsuccessfully; pathogenicity not yet determined.

Acid fast.—Negative. Agar 4 days.
Gram's stain.—Negative. Agar 3 days.
Flagella.—Polar, 1 to 2. Pitfield's stain.
Spores.—Negative.

TURNIP NO. 1.

Size 0.4 to 0.5 by 1.0 to 2.2μ. A common length is 1.6μ. Pathogenicity not yet established. Tests made on sugar beets failed.

Acid fast.—Negative. Agar 5 days.
Gram's stain.—Negative. Agar 2 days.
Flagella.—Polar, 1 to 2. Pitfield's stain.
Spores.—Negative.

TURNIP NO. 2.

Fresh isolation in July, 1910, from another gall on same plant, which had been kept alive in the hothouse. Growth on agar stroke resembled daisy; also the colony on agar plate. Not tested on plants.

Acid fast.—
Gram's stain.—
Flagella.—
Spores.—Negative.

SALSIFY.

Size 0.4 to 0.5 by 1.2 to 2.0μ. Rather slender, single, in pairs or 4's, end to end. Many of the rods are 1.5 to 1.8μ long. Beets inoculated unsuccessfully; pathogenicity not yet established.

Acid fast.—Negative. Agar 5 days.
Gram's stain.—Negative. Agar 3 days.
Flagella.—Unable to stain.
Spores.—Negative.

PARSNIP.

Size 0.4 to 0.8 by 1.0 to 2.4μ. Rarely as long as 2.4μ. Most about 1.2 to 1.5μ long, but frequently 1.8μ. A well-developed pair measured 3.2μ. Single, in pairs, or in 4's, rarely 8, end to end.[a] Rods frequently somewhat pointed at the ends. Beets inoculated unsuccessfully; pathogenicity not yet proved.

Acid fast.—Negative. Agar 5 days.
Gram's stain.—Negative. Agar 3 days.
Flagella.—Polar, 1 to 2. Pitfield's stain.
Spores.—Negative.

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[a] Short filaments were found in agar streaks when 19 days old.

TABULATED RESULTS OF INOCULATIONS.

TABLE II.—*Showing positive results of the pure-culture inoculations.*

Date of the inoculation.	Origin of culture.	Number and kind of plants inoculated.	Kind of culture used and its age.	Per cent which developed tumors.	Number and behavior of controls pricked.
Nov. 27, 1906	Daisy	7 daisy	1st agar sub., 3 d	100	21 plants inoculated with other organisms; free.
Dec. 13, 1906	do	8 daisy	1st agar sub., 16 d	100	None; at first only where inoculated.
Dec. 21, 1906	do	do	2d agar sub., 8 d	100	4; free.
Jan 8, 1907	do	do	Agar sub., 6 d [a]	100	None; at first only where inoculated.
Jan. 19, 1907	do	7 daisy	Agar sub., 5 d [b]	100	Do.
Feb. 6, 1907	do	8 daisy	2d agar sub., 9 d	100	4; free.
Feb. 18, 1907	do	9 daisy (12 shoots)	Agar sub., 7 d	[c] 100	1 plant, 50 punctures; also 72 check pricks on the 9 inoculated; all free.
Do	do	3 tomato	do	100	None; only where inoculated.
Do	do	3 tobacco	do	100	Do.
Mar. 2, 1907	do	18 potato	Agar	100	Several; free.
Do	do	12 carnation	Agar sub., 3 d	100	6; free.
Mar. 11, 1907	do	18 peach	Agar sub., 5 and 6 d	[d] 88 or 100	9; free, 180 punctures.
Mar. 20, 1907	do	6 tobacco	Agar sub., 2 d	100	1; free.
Mar. 27, 1907	do	6 potato	Agar sub., 3 d	100	None; only where inoculated.
Do	do	Rose	Agar sub., 2 d	100	3; free.
Mar. 29, 1907	do	Several cabbage	Agar sub., 4 d	100	None.
Apr. 3, 1907	do	8 American grape	Agar sub., 2 d	12	4; free.
Do	do	3 European grape	do	33	None.
Apr. 6, 1907	do	48 peach	Agar sub., 2 and 5 d	73	21 (420 punctures); free.
Apr. 8, 1907	do	18 hop	Agar sub., 4 d	100	8; free; more than 200 pricks.
Apr. 10, 1907	do	25 hop	Agar sub., 4 and 6 d	[e] 96 or 100	4; free.
Apr. 15, 1907	do	4 field daisy	Agar	[f] 100	1; free.
Apr. 17, 1907	do	12 sugar beet	Agar sub., 2 d	100	3; free.
Apr. 18, 1907	do	2 cabbage	Agar sub	100	Uninoculated; free.
Date (?)	do	Several turnip slices	Young agar sub	100	See text, p. 45.
Apr. 26, 1907	do	3 red beets, 4 carrots, 4 long radishes.	Agar sub., 2 d	100 100 100	None.
May 6, 1907	do	3 Jap. chrysanthemum	do	100	2; free.
Do	do	3 oleander	do	100	2; free.
May 9, 1907	do	9 European grape	Agar sub., 4 d	100	3; free.
May 14, 1907	do	do	Agar sub., 3 d	100	3; free.
July 23, 1907	do	6 Ch. coronarium	Agar sub., 5 d	100	No. ?— All free.
Do	do	6 Shasta daisy	do	100	Do.

[a] First from plate of December 18, 1906. [b] First from plate. [c] Every prick gave a gall; total, 102. [d] See text, p. 38. [e] See text, p. 49. [f] About 25 galls.

TABLE II.—*Showing positive results of the pure-culture inoculations*—Continued.

Date of the inoculation.	Origin of culture.	Number and kind of plants inoculated.	Kind of culture used and its age.	Per cent which developed tumors.	Number and behavior of controls pricked.
Aug. 1, 1907	Daisy	6 corn marigold	Agar sub., 2 d.	100	2; free.
Do.	do.	8 English daisy	do.	63	2; free.
Aug. 9, 1907	do.	8 European grape	Agar sub., 3 d.	75	3; free.
Sept. 19, 1907	do.	6 tobacco	do.	100	4; all free.
Sept. 26, 1907	do.	7 pyrethrum	Agar sub., 2 d.	100	3; free.
Nov. 15, 1907	do.	3 daisy	Agar sub., 4 d.	100	Check on next.
Do.	do.	24 sugar beet	do.	92	None. See below.
Nov. 18, 1907	do.	36 sugar beet	Agar sub., 3 d.	100	No specific checks held, but one side of the house was full of beets, and only the inoculated ones contracted the disease.
Do.	do.	6 daisy	do.	100	Checks on the preceding.
Feb. 11, 1908	do.	do	Agar sub., 2 d.	100	3; free.
Mar. 5, 1908	do.	4 oleander	do.	100	2; free.
Mar. 7, 1908	do.	6 oleander	do.	100	None; only where inoculated.
Do.	do.	3 European grape	do.	33	None.
Do.	do.	8 almond	do.	50	All uninoculated are free.
Mar. 12, 1908	do.	5 daisy	do.	100	None.
Do.	do.	10 oleander	do.	100	None; only where inoculated.
Do.	do.	4 clover	Agar sub., 3 d.	100	2; free.
May 25, 1908 May 26, 1908	do.	5 white poplar	Agar sub., 4 d.	20	Only where inoculated.
Do.	do.	8 Pterocarya	do.	37	Do.
Do.	do.	3 Persian walnut	do.	100	No other galls on tree.
June 9, 1908	do.	2 gray poplar	do.	50	Only where inoculated.
June 11, 1908	do.	5 sugar beets	Agar sub.	100	None.
Dec. 4, 1909	do.	8 sugar beets	Agar sub., 2 d.	100	House full of beets; only inoculated beets became diseased.
Nov. 8, 1909	Arbutus	16 sugar beet	do.	12	6; all free.
Dec. 1, 1909	Cotton	9 cotton	do.	44	3; all free.
Aug. 31, 1909	Grape	6 daisy	Agar sub., 4 d.	50	8; free.
Do.	do.	4 European grape	do.	50	
Sept. 7, 1909	do.	6 European grape	Agar sub., 3 d.	100	2; free.
May 7, 1910	do.	12 sugar beet	Agar sub., 1 d.	92	Only inoculated beets took the disease.
June 28, 1910	do.	12 almond	Young agar sub.	100	6; free.
June 14, 1909	Alfalfa	4 daisy	Agar sub., 4 d.	25	None.
Do.	do.	4 alfalfa	do.	a 100	Only where inoculated.
Do.	do.	2 sugar beet	do.	100	None.
July 16, 1909	do.	48 alfalfa	do.	25	12; free.
Do.	do.	5 sugar beet	do.	80	None; galls only where inoculated.
Sept. 7, 1909	do.	36 alfalfa	Agar sub., 3 d.	33	8; free.
Dec. 8, 1909	Alfalfa (Sept. 7)	Several young alfalfa	Agar pl., 6 d.	100	

Dec. 2, 1907	Peach	10 daisy	Colonies, 6 d.	100	4; free.
Dec. 4, 1907	....do	36 daisy	12 col. 9 d. and agar sub., 4 d.	92	600 + check pricks sterile.
Dec. 5, 1907	....do	6 peach	Agar sub., 3 d.	100	2; free.
Do.	Daisy	....do	....do	66	See preceding.
Jan. 13, 1908	Peach	60 peach	Agar sub., 2 d. (?)	92	36 (540 pricks) all free; also free, 900 check pricks on the inoculated trees.
Jan. 15, 1908	....do	6 rose	Agar sub., 1 d.	17	None.
Feb. 3, 1908	....do	10 daisy	10 col., 7 d.	100	None; only where inoculated.
Mar. 11, 1908	....do	3 apple (each several places)	Colonies, agar, 7 d.	100	Galls only where pricked.
Do.	....do	5 sugar beet	Agar sub.	100	Only where inoculated.
Mar. 24, 1908	....do	9 peach	Agar sub., 4 d.	66	4; free.
May 19, 1908	....do	13 apple	....do	b 38 or 100	Do.
May 20, 1908	....do	9 red raspberry	Agar sub., 2 d.	100	Do.
May 19, 1908; May 22, 1908	....do	34 black raspberry	Agar sub., 2 3, and 8 d.	97	Do.
June 10, 1908	....do	6 hop	Agar sub., 4 d.	100	2; free.
Oct. 13, 1908	....do	2 geranium (4 shoots)	Agar sub., 3 d.	100	Many other geraniums on same bench free.
Dec. 17, 1907	Rose	12 rose	Agar sub., 4 d.	17	3; free
Jan. 15, 1908	....do	....do	Agar sub., 1 d.	17	None.
Mar. 18, 1908	....do	8 daisy	Agar sub., 2 d.	100	1; free.
Dec. 3, 1908	....do	7 sugar beet	Agar sub., 7 d.	14	3; free.
Mar. 21, 1909	....do	3 daisy (each in 3 to 5 shoots)	Agar sub., 5 d.	50	2; free.
Jan. 27, 1909	Quince	8 daisy	Agar sub., 2 d.	25	Do.
Mar. 21, 1909	....do	3 daisy (9 shoots)	Agar sub., 5 d.	66	2; free.
Apr. 26, 1910	Beet	1 daisy (4 shoots)	Agar sub., 4 d.	25?	None.
Nov. 21, 1907	Hop	4 hop	Agar? sub., 2 d.	75	Do.
Feb. 8, 1908	....do	5 daisy (4 and 5 shoots on each).	Agar sub., 5 d.	c 100	Do.
Feb. 10, 1908	....do	12 daisy	12 col., 7 d.	17	Do.
Apr. 17, 1908	....do	6 sugar beet	Agar sub., 2 d.	100	Only inoculated plants gave tumors.
Apr. 25, 1908	....do	5 daisy	....do	100	None.
June 10, 1908	....do	8 hop	Agar sub., 4 d.	100	4; free.
Nov. 21, 1908	....do	3 tomato (36 groups of punctures).	Agar sub., 3 d.	100	2; free.
Apr. 16, 1909	....do	3 European grape	Agar sub., 2 d.	100	Do.
Do.	....do	3 almond	....do	66	None.
Mar. 7, 1910	Hop (after 26 transfers on media).	5 sugar beet	Agar sub., 3 d.	d 100	Other beets in same and adjoining rows remained free.
May 9, 1910	Hop	3 daisy (8 terminal shoots)	....do	33	None.
Nov. 12, 1910	....do	3 sugar beet	....do	100	Many plants in same bed.
Do.	....do	4 daisy	....do	75	Numerous daises on same bench picked at same time from col. 2 and 3 hop failed.
Nov. 30, 1910	....do	12 daisy	Beef-bouillon, 16 d.	33	
Dec. 2, 1910	....do	20 daisy	Agar sub., 2 d.	15	
Nov. 13, 1908	Old chestnut	1 daisy (4 shoots)	Colonies, 6 d.	e 100	None; galls only where inoculated.
Do.	....do	5 sugar beet	....do	100	None; galls only where pricked.
June 4, 1910	Flats poplar	10 sugar beet	Agar sub., 4 d.	100	Only where inoculated. An adjoining row, pricked at this time with new chestnut gall, failed and would also serve as a check.
June 10, 1910	....do	2 European grape	Young agar sub	100	Galls only where punctured.
May 9, 1910	Willow	3 daisy	....do	66	None; the galls appeared in the spots punctured.

a Including two-thirds of roots pricked. b Eight trees made no growth. c None well developed. d Galls large. e 4 galls, finally large.

TABLE II.—*Showing positive results of the pure-culture inoculations*—Continued.

Date of the inoculation.	Origin of culture.	Number and kind of plants inoculated.	Kind of culture used and its age.	Per cent which developed tumors.	Number and behavior of controls pricked.
Dec. 12, 1910	Willow	6 willow	3 d. agar sub	66	
Oct. 23, 1908	Apple	6 peach	Agar plate, 8 d	33	
Do	do	8 sugar beet	Agar sub., 2 d	25	
Oct. 22, 1908	Apple (soft), Washington	4 daisy	Agar col., 7 d., or 1st sub., 2 d	100	
Do	do	3 apple	Agar col., 7 d	66	None.
Nov. 9, 1908	Apple (hard), Iowa	8 daisy	Agar col., 5 d	50	8; on same plants; negative.
Nov. 18, 1908	do	3 daisy	Agar col., 9 d	100	No galls except where inoculated.
Dec. —, 1908	Apple (hard), Washington	Sugar beet	Agar	100	
Dec. 4, 1908	do	8 sugar beet	Agar sub., 2 d	25	None.
Nov. 13, 1908	Apple hairy-root (Iowa)	6 sugar beet	do	83	Do.
Dec. 22, 1908	Apple hairy-root (Washington).	11 sugar beet	Agar sub., 3 d	[a]90 or 100	Do.
Feb. 24, 1909	Apple hairy-root (New York).	7 sugar beet	Agar sub., 1 d	57	Number doubtful; all sound.
Apr. 5, 1909	do	8 apple seedlings	Agar sub., 2 d	[b]75	4; one galled.
May 21, 1909	Apple hairy-root	3 quince (90 punctures)	Agar sub., 2 d	100	Hard tissues pricked; galls did not appear for a long time.
Nov. 11, 1909	Apple hairy-root (New York)	8 sugar beets	Agar sub., 6 d	100	None.

[a] See text, p. 104.

[b] See text, p. 103.

213

TABLE III.—*Showing negative or doubtful results of inoculations.*

Date of the inoculation.	Origin of culture.	Number and kind of plants inoculated.	Kind of culture used and its age.	Per cent which developed tumors.	Remarks.
Jan. 6, 1907	Daisy	Several bulbs and leaves of common onion.	Agar sub., 4 d.	0	Slow growth.
Jan. 18, 1907	do.	7 daisy (24 places)	Agar sub., 49 d.	0	Culture probably dead.[a]
Feb. 14, 1907	do.	2 olive (several shoots each)	Agar sub., 7 d.	0	Probably not susceptible.
Mar. 2, 1907	do.	12 salsify	Agar sub., 3 d.	0	
Mar. 11, 1907	do.	1 olive	Agar sub.	0	Same culture successful on peach.
Mar. 12, 1907	do.	5 olive	Agar sub., 2 d.	0	Daisy inoculated from same culture gave knots.
Apr. 3, 1907	do.	18 rose	do.	0	Daisy controls took the disease.
Apr. 11, 1907	do.	2 European grape	Agar sub. 5 d.	0	One dead; other no growth.
Do.; Apr. 12, 1907	do.	34 blackberry	Agar sub., 5 and 6 d.	0	Many died; others made a moderate growth.
Do.	do.	29 raspberry	Agar sub., 6 d.	35?	Rejected because the checks also contracted the disease.
Apr. 13, 1907	do.	6 apple	Agar culture	50?	Rejected because some had hairy root when inoculated.
Apr. 17, 1907	do.	6 walnut	Agar sub., 5 d.	0	Slow growth.
Do.	do.	5 gray poplar	do.	0	Do.
Do.	do.	6 Lombardy poplar	do.	0	Inoculated on roots.
Do.	do.	6 cottonwood	do.	0	Slow growth.
June 9, 1907	do.	1 European grape	Agar sub., 4 d	0	Plant died.
Aug. 9, 1907	do.	11 common fig	Agar sub., 3 d.	0	Probably not susceptible; produced galls on grape.
Dec. 19, 1907	do.	7 daisy	Agar sub., 2 d.	0	Plants not growing.
Mar. 7, 1908	do.	3 common fig	do.	0	
Do.	do.	3 cabbage	do.	0	
Do.	do.	3 chestnut	do.	0	
May 26, 1908	do.	1 Impatiens sultana (several places).	Agar sub., 4 d	0	
Do.	do.	5 scarlet clover	do.	0	Dwarfed old plants growing very slowly.
Do.	do.	5 apple	do.	0	
Do.	do.	3 Lombardy poplar	do.	0	Inoculated in rapidly growing shoots.
Apr. 4, 1910	do.	11 onion	Young agar sub	0	Newest isolation.
Do.	do.	2 Impatiens sultana[b]	do.	0	Culture actively pathogenic to daisy.
Apr. 6, 1910	do.	12 common fig	do.	0	Recent isolation from daisy.
Apr. 7, 1910	do.	10 chestnut	do.	0	Newest isolation.
Do.	do.	8 red oak	Agar sub.	0	Shoot grew only 1 inch after inoculation.
Apr. 14, 1908	Honeysuckle	8 daisy	Agar sub., 4 d.	0	Possibly the wrong organism.
Nov. 2, 1909	Arbutus	5 daisy	Agar sub., 5 d.	0	Young growing plants.
May 7, 1910	do.	6 daisy	Young agar sub.	0	
Nov. 11, 1909	Cotton	9 sugar beet	Agar sub., 5 d.	0	Young growing plants.
Nov. 16, 1909	do.	12 daisy	do.	0	Plants in good condition.

[a] For additional failures see page 181.

[b] One (red flowered) 5 places; 1 (white flowered) 7 places.

213

TABLE III.—*Showing negative or doubtful results of inoculations*—Continued.

Date of the inoculation.	Origin of culture.	Number and kind of plants inoculated.	Kind of culture used and its age.	Per cent which developed tumors.	Remarks.
Apr 29, 1910	Cotton	7 daisy	Agar sub., 2 d	0	
July 5, 1910	do	6 sugar beet	Agar sub., 4 d	0	Plants grew very slowly.
Mar. 27, 1908	Grape	3 almond	Agar sub., 2 d	0	Suspect wrong organism.
Mar. 28, 1908	do	16 daisy	Agar sub., 3 d	0	Do.
Aug. 31, 1909	do	3 sugar beet	Agar sub., 4 d	0	Same cultures were partially successful on grape and daisy.
June 16, 1909	Alfalfa	2 alfalfa (8 roots)	Agar col., 6 d	0	Dwarfed old plants.
Dec. 8, 1909	do	Several old alfalfa	Young agar sub	0	Plants in poor condition.
Jan. 27, 1910 } Feb. 1, 1910 }	do	14 peach	Agar sub., 1 d	0	Trees were beginning to leaf.
Jan. 16, 1908	Peach	6 apple	4th agar sub., 1 d	0	Trees dormant.
Jan. 23, 1908	do	10 apple	5th agar sub., 2 d	50?	Rejected because checks also contracted disease.
Mar. 11, 1908	do	2 daisy	Agar col., 7 d	0	Possibly wrong colonies used; apple took disease from other colonies on same plate.
Do	do	2 olive	do	0	
May 22, 1908	do	35 apple	Agar sub., 3 d	(?)	Mixed with checks in planting?
Oct. 13, 1908	do	2 walnut	do	0	Shoots hard when inoculated.
May 6, 1909	do	4 peonia	Agar sub., 2 d	0	
May 18, 1909	do	11 phlox	Agar sub., 4 d	0	
Do	do	10 verbena	do	0	
Jan. 14, 1908	Rose	8 peach	Agar sub., 1 d	0	
Jan. 23, 1908	do	6 apple	Agar sub., 2 d	0	Trees dormant.
Mar. 18, 1908	do	6 rambler rose	do	0	
May 9, 1909	do	25 peach	Agar sub., 3 d	0	Trees not in best condition.
July 9, 1907	Raspberry	4 daisy	Colonies, 7 d	0	Possibly wrong organism used.
Feb. 26, 1909	Quince	7 sugar beet	Agar sub., 3 d	0	Beets made slow growth.
May 14, 1909	do	8 quince	Agar?	0	Material not suitable.
May 21, 1909	do	3 quince	Agar sub., 2 d	0	
Mar. 9, 1910	do	7 quince	do	0	
July 2, 1910	do	16 sugar beet	Young agar sub	0	Beets made slow growth.
June 27, 1910	Sugar beet	10 sugar beet	do	0	Probably of wrong organism; beets made slow growth.[a]
Apr. 17, 1908	Hop	4 daisy	Agar sub., 2 d	0	
May 6, 1909	do	3 peonia	Young agar sub	0	
May 9, 1910	do	10 olive	do	0	Shoots growing very rapidly; many pricks. Infectious to beet.
May 24, 1910	Old chestnut	5 sugar beets	do	0	
June 10, 1910	New chestnut	5 sugar beets and 1 European grape.	do	0	
Jan. 23, 1908	Apple (hard), Washington	10 apple	Agar sub., 2 d	0	All from 1 colony; probably wrong organism.
Feb. 24, 1908	do	6 daisy	3d agar sub., 4 d	0	Probably wrong organism.

Do	do	3 apple	Agar sub., 4 d	0	Organism from same set of plates as preceding.
Nov. 9, 1908	Apple (hard), Iowa	2 pelargonium	Agar col., 5 d	0	Daisies contracted disease.
Nov. 12, 1908	do	4 apple	Agar col., 8 d	0	Trees growing very slowly; daisies gave galls.
Do	do	9 sugar beet	do	0	Plants nearly dormant.
Do	do	6 root tips of Monstera	do	0	
Dec. 4, 1908	Apple (hard)	Several plants of tomato	Agar sub., 2 d	0	Inoculated on well-developed stem roots.
June 24, 1910	do	10 pelargonium	do	0	
Do	Old apple	10 sugar beet	Young agar sub	0	See "Morphology;" probably an intruder.
Nov. 21, 1908	Apple, hairy-root	3 tomato (36 groups of punctures).	Agar sub., 3 d	0	Plants full grown; inoculated on stem roots.
Dec. 10, 1908	do	Tomato (several much younger plants).	do	0	
Apr. 1, 1909	do	10 tomato	Agar sub., 2 d	0	See text.

[a] For additional failures, see remarks p. 85.

213

CULTURAL CHARACTERS.

EXPLANATORY STATEMENT.

Such comparative studies as we have been able to make are included in the following tables and memoranda. Many of them were made or repeated in the spring and summer of 1910 during the preparation of this bulletin.

In offering these incomplete data it may be pointed out that probably some errors have slipped into these records, as the time was not sufficient for exhaustive tests of all the cultural characteristics of all these strains and for the elimination of all possible intruders through repeated poured plate separations and further inoculations. It is the more likely that some errors are included owing to the fact that in 1910 a number of our forms had ceased to be pathogenic, e. g., peach, chestnut, apple, quince; but whether this was due only to loss of a peculiar quality, or to the right organisms having been driven out of our cultures by unobserved intruders was not determined beyond all doubt, except that clearly the "old apple" appeared to be something entirely different from what we had on the start, and very probably the quince and the sugar beet.

To straighten out fully all the tangle of interrelations here touched upon would require so many additional months of work that it has appeared best to publish at once what we have, leaving the unsettled problems for further study.

GROWTH ON AGAR.

When these organisms were grown for 3 days at 23° to 25° C. upon slant +15 peptonized beef agar containing 1 per cent agar flour, and inoculated by needle stroke from 18-day-old slant agar cultures, there was in each case a well-developed shining white streak and some growth in the condensation water. The agar was not stained. Slight differences not easily definable were visible, the most pronounced of which were the following:

(1) White watery translucent streaks: Newest daisy, arbutus, new apple, apple hairy root, new chestnut, grape, alfalfa (the last showing transitions to 2). There were no crystals, or only a trace (new apple).

(2) Similar streaks but whiter, i. e., less translucent: Old daisy, peach, hop, old rose, new rose, beet, cotton. Numerous prismatic crystals, except in beet which had only a few.

(3) White shining flat growth, i. e., thinner than in the preceding and trace of crystals: Old apple. The growth of this when first isolated (2 years ago) was like No. 1, i. e., translucent watery. (See under *Morphology*, p. 129.)

(4) A thin white growth inclined to wrinkle, crystals few and large: Quince. This is the only culture showing any wrinkles.

This experiment was repeated in July, 1910, at a higher temperature (30° to 31° C.), inoculating from younger cultures (1 to 3 day agar), with less typical growth (in some cases colony wise) but nevertheless with essentially the same results. Under group 2 fewer with crystals. The old apple looks decidedly unlike the others and is probably an intruder.

GROWTH IN +15 BEEF BOUILLON WITH 1 PER CENT WITTE'S PEPTONE.

At the end of 3 days at 23° to 25° C., inoculating from slant agar cultures 18 days old, the appearances in test tubes containing 10 c. c. of the fluid were as follows:

(1) Incomplete easily fragmenting pellicle, fluid nearly clear, stringy threads on shaking: Arbutus, alfalfa, newest daisy.

(2) Cloudier and with a heavier pellicle but otherwise like 1: New rose, old rose, cotton, hop, old daisy (B), peach.

(3) Cloudy and more or less stringy but destitute of pellicle, some precipitate: New apple, old apple, apple hairy-root, beet.

(4) Cloudy with some flocks, but no pellicle or strings: Quince.[a]

This experiment was repeated in July, 1910, at 30° to 31° C., inoculating from 3-day-old bouillon cultures. The results at the end of 3 days were the same, except that now the newest daisy had no pellicle, and the old rose an easily fragmenting pellicle. The previously untried strains fell into the above-named groups as follows:

Group 1.—Newport poplar No. 1, salsify.
Group 2.—Turnip No. 1.
Group 3.—Grape, Newport poplar No. 2.
Group 4.—New chestnut, willow.

The parsnip did not resemble the others. It was heavily and uniformly clouded with a precipitate which rose in a swirl on shaking. There were no strings, rim, or pellicle.

CANE-SUGAR PEPTONE WATER.

When grown for 18 days in river water containing 2 per cent Witte's peptone and 2 per cent c. p. cane sugar the strains did not brown the fluid, but behaved as follows:

(1) Fluid clear and not much precipitate or flocculence, but a very copious thick white pellicle (0.5 to 1.5 cm. thick, mostly the latter): Alfalfa, turnip No. 1, Flats poplar, new rose (pellicle 0.5 cm.) cotton, hop, peach, old daisy, newest daisy.

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[a] The old chestnut was discarded as contaminated, and the grape did not grow.

(2) Fluid feebly clouded, some strings and flocks, pellicle incomplete, thin, fragmenting, not much precipitate: Salsify, parsnip, arbutus, grape, apple hairy-root, new apple, Newport poplar No. 1.

(3) Thin cloudy, no rim, no pellicle, but a precipitate which makes the fluid cloudy on shaking: Beet.

(4) Trace of rim, no pellicle, no strings, moderate clouding, slight precipitate: Willow.

(5) Moderately cloudy, trace of rim, no pellicle, quite cloudy on shaking, but not much flocculence, and no strings: New chestnut.

(5a) Moderate white rim and very scanty pellicle, quite cloudy with much coarse flocculence on shaking, but no strings: Newport poplar No. 2.

(6) Fluid clear, no rim, no pellicle, no strings, or filaments; thinly clouded on shaking by a great number of fine pseudozooglœæ: Old apple.

(7) Like 6, but clouds on shaking with a finer precipitate: Quince.

At the end of a month the tubes still fell into the old groups and none of the fluids were brown stained. When tested with neutral litmus paper the cultures gave the following reactions:

Group 1.—All strongly alkaline except alfalfa (slightly alkaline) and newest daisy (neutral or slightly acid).
Group 2.—Much less growth: New apple (slightly acid); apple hairy-root and arbutus (neutral); grape (alkaline); Newport poplar No. 1 (alkaline); parsnip (alkaline); salsify (neutral).
Group 3.—Beet (strongly alkaline).
Group 4.—Willow (strongly alkaline).
Group 5.—New chestnut (slightly alkaline).
Group 5a.—Newport poplar No. 2 (strongly alkaline).
Group 6.—Old apple (2 tubes, plainly acid).
Group 7.—Quince (2 tubes, alkaline).

Only groups 1 and 2 contained organisms of recently proved virulence. Subsequently the willow was proved to be pathogenic to willow.

MALTOSE PEPTONE WATER.

When grown for 3 months in river water containing 2 per cent Witte's peptone and 1 per cent maltose the strains behaved as follows:

(1) Fluid clear, unstained, slight stringy rim, moderate flocculent precipitate which clouds fluid on shaking: Newest daisy (neutral to litmus), alfalfa (strongly alkaline), arbutus (acid to litmus), apple hairy-root (strongly alkaline).

(2) Like 1, but fluid brownish and alkaline: Grape.

(3) Clear unstained fluid strongly alkaline to litmus, pellicle 0.5 cm. thick, slight precipitate: New rose.

(4) Fluid dried out one-third. Dense white slimy pellicle nearly filling the remainder of the fluid, i. e., 2 cm. deep, not much precipitate, fluid alkaline: Old daisy, hop.

(5) Exactly like the preceding except that fluid and pellicle are slightly brownish, fluid alkaline: Peach, cotton.

(6) Thinly clouded, no rim, or pellicle, fluid unstained, a moderate precipitate which shakes up in a coarse flocculence, which is most abundant in the beet: Old apple (acid to neutral litmus paper), beet (strongly alkaline), old chestnut (neutral to litmus).

(7) Fluid moderately cloudy and yellowish (alkaline to litmus), rim yellowish white, not stringy, no pellicle, moderate precipitate which shakes up in coarse flocculence: Quince.

TABLE IV.—*Showing behavior*[a] *of crown-gall organisms in peptonized beef bouillon of varying grades of alkalinity or acidity.*

[Inoculated from 3-day-old and 7-day-old peptonized bouillon cultures, except −25, which was from 19-day-old beef bouillon. Examined at end of 28 or 31 days, except −25, which was 9 days old. The +34 was acidulated with citric acid, the +38 with malic; the alkali was sodium hydroxide.]

Organism.	Titration (grade of alkalinity or acidity).		
	−34	−25	−24
Newest daisy	0, 0	0, 0, 0	3, 3, 3.
Old daisy	2	3, 3 [b]	3 [b].
Peach	3		3 [b]
Hop	3	3 [b]	4 Ragged pellicle.
New rose	0	2 Thin pellicle	3 Good pellicle.
Old apple	0	0	0.
Apple hairy-root	0	0	0.
Alfalfa	0	1	0.
Grape	0	1 or 2	0.
Chestnut	0	3 Uniformly cloudy. Cont.?	3 Uniformly cloudy Cont.?
Arbutus	0	0	0.
Cotton	3	3 Thin pellicle	3.
Quince	2	2?	0.
Beet	0	2	2 or 3.

Organism.	Titration (grade of alkalinity or acidity).		
	−16	+34	+38
Newest daisy	2	3 Thin firm pellicle; clear fluid.	0.
Old daisy	3 [b]	5 [b]	4 [b] Clear fluid.
Peach	4 [b]	5 [b]	5 [b] Fluid cloudy.
Hop	4 Ragged pellicle	5 [b]	5 [b].
New rose	3 Ragged pellicle	0	0.
Old apple	0	3 Uniformly cloudy. Cont.?	4 Rim and cloudy fluid.
Apple hairy-root	0	0	0.
Alfalfa	0	3 White rim with precip.	0.
Grape	0	3	2 Strings and feeble clouding.
Chestnut	3 Uniformly cloudy. Cont.?	0	0.
Arbutus	0	2 Thin white rim	0.
Cotton	4 Fragmenting pellicle	5 Fragmenting pellicle	5 Heavy pellicle, breaking on hard shaking.
Quince	0	0	0.
Beet	3 Strings, precipitate pale salmon.	0	0.

[a] Explanation of figures indicating growth: 0=no growth; 1=trace; 2=slight; 3=moderate; 4=good; 5=copious.

[b] Thick firm pellicle, not broken by shaking.

Some additional tests were made in 1910 in acid and alkaline peptonized beef bouillon with the results shown in Table V.

TABLE V.—*Showing behavior [a] of crown-gall organisms in bouillons of varying reaction, the records being taken some weeks after inoculation.*

[The alkali was sodium hydrate.]

Organism. (The first four strains are of most recently established virulence.)	Titration (grade of alkalinity or acidity).				
	−29	−25	−23	+36 (citric acid).	+34 (malic acid).
Flats poplar	0	4	2	4	4
Hop	4	4	.....	.....	.....
Grape	0	0	2	.....	.....
Peach	4	4	.....	.....	.....
Apple hairy-root	.....	.....	0	0	0
Old apple	.....	.....	4	.....	.....
Chestnut	.....	.....	0	4	4
Alfalfa	.....	.....	4	4	4
Quince	.....	.....	2 or 0	0	0
Beet	.....	.....	4	0	0
Arbutus	.....	.....	2 or 0	0	0
New rose	.....	.....	.....	0	0

[a] Explanation of figures indicating growth: 0=no growth; 2=slight; 4=good, with pellicle.

TABLE VI.—*Showing behavior [a] of crown-gall organisms in +15 bouillon containing sodium chloride at room temperatures.*

[Transfers from 5-day-old bouillon cultures.]

Organism.	Time (days).		
	4.	15.	31.
Daisy: [b]			
3.0 per cent	0	0	0
3.5 per cent	0	0	0
4.0 per cent	0	0	0
Peach:			
3.0 per cent	2	3	3
3.5 per cent	0	0	0
4.0 per cent	0	0	0
Hop:			
3.0 per cent	2	5	5
3.5 per cent	2	2	2
4.0 per cent	0	0	0
Rose:			
3.0 per cent	2	5	5
3.5 per cent	0	0	0
4.0 per cent	0	0	0
Apple:			
Old strain—			
3.0 per cent	3	5	5
3.5 per cent	4	5	5
4.0 per cent	2	5	5
New strain—			
3.0 per cent	0	0	0
3.5 per cent	0	0	0
4.0 per cent	0	0	0
Apple hairy-root:			
3.0 per cent	0	0	0
3.5 per cent	0	0	0
4.0 per cent	0	0	0
Alfalfa:			
3.0 per cent	0	0	0
3.5 per cent	0	0	0
4.0 per cent	0	0	0

Organism.	Time (days).		
	4.	15.	31.
Grape:			
3.0 per cent	0	0	0
3.5 per cent	0	0	0
4.0 per cent	0	0	0
New chesnut:			
3.0 per cent	.....	0	.....
3.5 per cent	.....	0	.....
4.0 per cent	.....	0	.....
Old chestnut:			
3.0 per cent	0	0	2
3.5 per cent	0	0	0
4.0 per cent	0	0	0
Arbutus:			
3.0 per cent	0	.....	0
3.5 per cent	0	.....	0
4.0 per cent	0	.....	0
Cotton: [c]			
3.0 per cent	2	5	5
3.5 per cent	1	1	1
4.0 per cent	0	0	0
Beet:			
3.0 per cent	3	.....	5
3.5 per cent	3	.....	4
4.0 per cent	2	.....	4
Quince:			
3.0 per cent	0	0	0
3.5 per cent	0	0	0
4.0 per cent	0	0	0
Flats, poplar:			
3.0 per cent	.....	0	.....
3.5 per cent	.....	0	.....
4.0 per cent	.....	.....	.....

[a] Explanation of figures indicating growth: 0=no growth; 1=very slight; 2=slight; 3=fairly good. 4=good; 5=heavy.
[b] In a repetition of daisy there was moderate growth at end of six weeks in 3 and 3.5 per cent.
[c] In a repetition of cotton there was no growth at end of three days and at end of fifteen days; at end of forty days the growth was 3, 3, and 0, respectively, for 3, 3.5, and 4 per cent.

TABLE VII.—*Showing behavior*[a] *of crown-gall organisms in Cohn's solution at room temperature (20° to 30° C.).*

[Transfers from 4-day-old cultures.]

Organism.	Date of examination.						Remarks.
	3 days.	1 week.	13 days.	2 weeks.	24 days.	6 months.	
	Growth.	Growth.	Growth.	Growth.	Growth.	Growth.	
Daisy	0	0	....	0	....	....	
Peach	0	2	....	2	....	4	Cloudy; numerous flocks.
Hop	0	2	....	b 3	....	4	Faint clouding; stringy precipitate.
Rose	0	2	....	2	....	4	Many flaky strings.
Apple	0	0	....	0	....	0?	No clouding; some small fine flocks.
Apple hairy-root	0	1	....	3	....	4	Milky cloudy; membranous precipitate visible on shaking.
Alfalfa	0	1	....	1	....	3	Cloudy; some flocks.
Grape	0	1	....	1	....	4	Cloudy flocculent; clumps of branched crystals.
New chestnut	....	....	(c)	....	....	....	
Old chestnut	0	2	....	2	....	3	Milky cloudy; no flocks or precipitate.
Arbutus	0	0	....	0	0	....	
Beet	0	0	....	0	....	....	
Cotton	0	0	....	0	0	....	
Quince	0	2	....	2	....	5	Yellowish stain.
Flats poplar	....	....	d 0	....	....	....	

a Explanation of figures indicating growth: 0=no growth; 1=very slight; 2=slight; 3=fairly good; 4=good; 5=heavy.

b Stringy growth.

c One tube milky cloudy; one tube clear.

d Two tubes, no growth; the same stock grew in nitrate bouillon.

TABLE VIII.—*Showing behavior* [a] *of crown-gall organisms on starch jelly at room temperature.*

[Transfers from 5-day-old agar cultures.]

Organism.	First test.			Repetition.			
	3 days.	11 days.		8 days.		34 days.	
	Growth.	Growth.	Remarks.	Growth.	Remarks.	Growth.	Remarks.
Daisy:							
Old strain	5	5	Color unchanged	3	(b)	3	(c)
Newest strain	....	....	....	1	(d)	1	(e)
Peach:							
Old strain	5	5	Light brown	3	(b)	3	(c)
Another strain	5	5	Color unchanged	....	....	....	
Hop	5	5	Medium gray	3	(f)	3	No diastasic action; no brown stain.
Rose:							
Old strain	5	5	Medium light brown.	3	(b)	3	(c)
New strain	....	....	....	3	(f)	3	No diastasic action; no brown stain.
Apple:							
Old strain	1	3	Color unchanged	1	(d)	1	(e)
New strain	1	3	do	1	(d)	1	(e)
Apple hairy-root	1	3	do	1	(d)	1	(e)
Alfalfa	3	3	do	1	(d)	1	(e)
Grape	3	4	do	1	(d)	1	(e)
New chestnut	1	3	do	3	Same as 34 days.	3	No diastasic action; no brown stain.
Arbutus	....	....	....	1	(d)	1	(e)
Beet	....	....	....	5	(g)	....	
Cotton	5	5	Light brown	3	(b)	3	(c)
Quince	1	5	Color unchanged; liquefaction.	3	(f)	3	No diastasic action; no brown stain.
Flats poplar	....	....	....	....	....	[b] 3	Brownest of all.[h]

[a] Explanation of figures indicating growth: 1=scant; 3=moderate; 4=good; 5=copious.

[b] Diastasic action feeble or absent. A brownish stain mixed with the white. In cotton the original streak is brownish, but the young growth pushing out to either side of the old is white. In old rose the base of streak is white, upper three-fourths is brownish, but beyond the brown the new growth is white The margin of old daisy streak is also whitish.

[c] Old daisy, old rose, peach, and cotton look alike—decided browning of the slime in each case, and a paler (brown) staining of the body of the jelly. No diastasic action and not a very copious growth. The margin of streak in cotton and base in old rose, which were white at end of 8 days are now brownish, while the older portions have become dark brown.

[d] No indication of any diastasic action. Purest white growth is that shown by alfalfa.

[e] No diastasic action; only slight increase in growth. A little increase in color toward cream, and in grape toward brownish.

[f] Diastasic action absent or scanty. More color in slime which approximates a pale cream.

[g] Abundant salmon-colored growth which has run down and filled the V. On plating out a pinkish intruder was discovered.

[h] In Flats poplar, recently tested on sugar beet and found to be virulent, there was at the end of 30 days a decided brownish stain throughout the medium (Ridgway's drab to drab gray).

TABLE IX.—*Showing indol reaction of crown-gall organisms*[a] *in two media at room temperature.*

[Transfers from 5-day-old bouillon cultures.]

Organism.	Uschinsky's solution + Witte's peptone.		2 per cent Witte's peptone in water.		
	3 days.	10 days (no change on heating).	24 hours.	26 days.[b]	33 days.
Daisy:					
Newest	0	0	0	Distinct	
Old strain	...	...	...	Feeble	
Peach:					
Strain 1	0	0	0	Distinct	
Strain 2	0	...	...	...	
Hop	0	0	0	Feeble	
Rose:					
Old strain	0	0	0	...	
New strain	...	...	...	Feeble	
Apple	0	0	0	0	
Apple hairy-root:					
Old strain	0	} Faint pink	0	(c)	
New strain	0	}			
Alfalfa	0	0	0	Feeble	
Grape	0	Faint pink	0	Distinct; good as daisy.	
Chestnut:					
New	...	...	...	...	Moderate on heating.
Old	0	0	0	0	
Arbutus	...	...	0	Feeble	
Beet	...	...	0	(?)	
Cotton	0	Faint pink	0	Distinct	
Quince	0	0	0	0	
Flats poplar	...	...	...	...	Distinct.

a Tested by adding to each tube 1 c. c. of 1 : 200 sodium nitrite and 10 drops of sulphuric acid.
b Positive reaction only after heating to 80° C., except daisy and trace in peach and Flats poplar.
c No growth.

Owing to Brizi's statements respecting the rapid production of indol by his *Bacillus populi* additional tests for indol were made in river water containing 2 per cent Witte's peptone, using Flats poplar, the virulence of which had been recently established:

(1) Tube copiously inoculated and incubated 24 hours at 30° C., which gave an unusually heavy growth. Result: No trace of color on adding reagents, and none, or merest trace, on heating for five minutes at 80° C.

(2) On July 26, 1910, the above experiment was repeated. Several tubes were inoculated copiously and incubated at 30° C., the growth being prompt and typical. They were tested as follows, using for each tube 1 c. c. of sodium nitrite water (1 : 200) and 10 drops of sulphuric acid.

July 27 (28 hours): Considerable growth but no trace of red color at first, nor after some minutes, nor on heating at 80° C. for 4 minutes.

July 28 (50 hours): Growth has increased. No trace of red color cold, and none on heating for 5 minutes at 80° C.

July 29 (74 hours): Good growth continues. No red color on adding reagents cold. A trace (?) on heating at 80° C.

This experiment was repeated in October, 1910, with the same result: In 48 hours, no reaction either cold or on heating, growth good in form of shreds and strings; 83 hours, no reaction cold; on heating 5 minutes at 80° C., doubtful or possibly a trace of pink. The test was made in both 1 and 2 per cent peptone water.

REDUCTION OF NITRATES.

PRELIMINARY STATEMENT.

As already pointed out, the daisy organism does not reduce nitrates.

It is probable also that none of the others reduce nitrates, in the ordinary meaning of the term, but some doubt still exists.

Early tests by the junior writer having led to contradictory results (in some instances), further experiments were made in 1910, but not enough wholly to clear up the situation. The following is a summary of all our experiments:

DAISY.

(1) *Several old tests by Doctor Smith*—all negative.

Old tests by Miss Brown.—(2) 13 days, negative; (3) 5 days, both old and newest strains, negative; (4) 33 days, negative.

Tests in 1910 by Doctor Smith.—(5) 69-day-old cultures by Miss Brown in her nitrate bouillon No. 600. Old daisy, 2 tubes (strongly alkaline to litmus): Trace of blue in bottom which disappears on shaking; reagents pure, i. e., tested. Newest daisy: Trace of blue in bottom which shakes out. The checks also give a purple reaction in bottom of tube but not if the starch is withheld. The starch was then suspected and retested, but on adding KI water and H_2SO_4 to 20 c. c. of the boiled starch no blue reaction whatever was obtained.

PEACH.

Old tests by Miss Brown.—(1) 13 days, reduced; (2) 5 days, slight reduction; (3) 33 days, reduced.

Tests in 1910 by Doctor Smith.—(4) 69-day-old culture by Miss Brown in her bouillon No. 600 (culture strongly alkaline to litmus), copious reduction; does not shake out; check tubes give some blue reaction at bottom which goes on shaking; reagents pure. (5) Plates poured and portions of 8 colonies transferred to 8 tubes of bouillon; strong growth. Tested after 7 days, all negative, i. e., 4 failed, and 4 showed traces of blue at bottom in the bacterial precipitate, which color disappeared on shaking. Check tubes showed no nitrite present. The cultures were then tested for nitrate (H_2SO_4 and diphenylamine) with customary blue reaction. (6) Sec-

ond set of plates poured and portions of 11 typical-looking colonies transferred to as many tubes of the bouillon. Tubes tested on third day, when well clouded, all negative. These tubes foamed on shaking; the check tube did not. Before the reagents were added, the fluid in the check tube was neutral (or nearly so) to litmus, and that in the inoculated tubes strongly alkaline.

HOP.

Old tests by Miss Brown.—(1) 13 days, negative; (2) 5 days, reduced; (3) 33 days, trace of blue which disappeared on shaking.

Tests in 1910 by Doctor Smith.—(4) 69-day-old culture by Miss Brown in her nitrate bouillon No. 600 (fluid strongly alkaline to litmus). On adding the reagents, a trace of blue in bottom, which shakes out.[a]

ROSE.

Old tests by Miss Brown.—(1) 13 days, reduced; (2) 5 days, reduced (both new and old strains); (3) 33 days, reduced (both strains).

Tests in 1910 by Doctor Smith.—(4) 69-day-old culture by Miss Brown in her nitrate bouillon No. 600: New rose—Copious reduction; does not shake out. Old rose (strongly alkaline to litmus)—Copious blue reaction, does not shake out; reagents tested and found pure. (5) New rose—Plates poured and portions of 10 colonies transferred to as many tubes, also 3 tubes inoculated from mixed colonies; tubes tested when 5 days old and fluid well clouded; one check tube, negative; of 10 tubes, 7 gas forming, 3 non-gas forming; the latter did not reduce; the former showed trace of blue on pellicle where acid fell; of the 3 tubes from mixed colonies, 1 reduced, 2 did not.[b] (6) Old rose—Plates poured and portions of 8 colonies transferred to as many tubes of bouillon; two checks held; tests after a week; fluid heavily clouded and contamination suspected. Result—Checks negative; of the inoculated tubes, 4 show a trace of blue on the bottom, which disappears on shaking, 2 are heavily blued throughout, and 2 are inter-

[a] A fresh isolation from hop made in 1910, and pathogenic to daisy and sugar beet, was tested as follows for nitrate reduction:

Five tubes at end of 7 days' growth (colony 1), negative. Two tubes of same lot at end of 27 days gave a very strong reduction. Contamination was then suspected and poured plates were made. At the end of 3 days 12 colonies were transferred to nitrate bouillon. These cultures were tested at the end of 10 days, when the bouillon was well clouded. Ten gave no reduction, 1 gave a slight color which shook out, 1 reduced moderately, color persisting. Transfers from these 12 tubes were made to plain bouillon before testing, and from these bouillon cultures other 12 tubes of nitrate bouillon were inoculated on January 27 and tested at end of 14 days, when all were moderately clouded and bore a heavy pellicle which fragmented easily on shaking. The result on adding 10 drops of boiled starch water, 1 c. c. of 1 ; 250 fresh potassium iodide water, and 5 drops of 2 : 1 sulphuric acid water were as follows: Two tubes, no reduction; 10 tubes, blue reaction in precipitate (fallen pellicle), in two of these there was also a trace of blue at the top of the fluid. The mass of the fluid was entirely free from blue color, and all of the color disappeared on shaking.

[b] Growth on agar slant August 3, 1910, looked wrong, i. e., it was pinkish white. Examined after 19 days.

mediate, i. e., one blue at bottom, shaking out, the other remaining pale blue after shaking. (7) Old rose—Second set of plates poured and portions of 11 colonies transferred to as many tubes; cultures tested on third day, when well clouded. Result—Check negative; 11 inoculated tubes all blue at bottom in bacterial precipitate; 1 tube remained pale blue after shaking; the other 10 became colorless; on shaking, the inoculated tubes became half filled with foam.

Conclusion: New rose, contaminated; old rose, doubtful.

APPLE.

Old tests by Miss Brown.—(1) 13 days, reduced; (2) 5 days, old apple, reduced; new apple, negative, possibly no growth; (3) 33 days, old apple, reduced; new apple, trace (?), gave scarcely any growth.

Tests in 1910 by Doctor Smith.—(4) 69-day-old culture by Miss Brown in her bouillon No. 600 (fluid strongly alkaline), copious blue reaction which does not shake out; the new apple gave no growth.

APPLE HAIRY-ROOT.

Old tests by Miss Brown.—(1) 13 days, negative; (2) 5 days, reduced; (3) 33 days, trace(?), scarcely any growth.

Tests in 1910 by Doctor Smith.—(4) Plates poured and portions of 12 colonies transferred to as many tubes of the bouillon. The tests were made at the end of 13 days. One tube is cloudy with a moderate rim and precipitate, but the others show scarcely any growth. Result: All negative; no reduction.

ALFALFA.

Old tests by Miss Brown.—(1) 13 days, reduced; (2) 5 days, reduced; (3) 33 days, trace of blue which disappears on shaking.

Tests in 1910 by Doctor Smith.—(4) 69-day-old culture by Miss Brown in her bouillon No. 600—blue in bottom on adding the reagents (color shakes out). (5) Plates poured and portions of 12 colonies transferred to as many tubes of the bouillon; cultures tested when 5 days old and well clouded; check, negative; 12 cultures, all negative.

GRAPE.

Old tests by Miss Brown.—(1) 13 days, reduced; (2) 5 days, negative; (3) 33 days, negative.

Tests by Doctor Smith in 1910.—(4) 69-day-old culture by Miss Brown in her nitrate bouillon No. 600, moderate growth, negative.

OLD CHESTNUT.

Old tests by Miss Brown.—(1) 13 days, reduced; (2) 5 days, negative; (3) 33 days, negative.

Tests in 1910 by Doctor Smith.—(4) 69-day-old culture by Miss Brown in her bouillon No. 600, negative; growth abundant.

ARBUTUS.

Old tests by Miss Brown.—(1) 5 days, reduced; (2) 33 days, trace of color, scarcely any growth.

Tests by Doctor Smith in 1910.—(3) Plates poured and portions of 9 colonies transferred to as many tubes of the nitrate bouillon; tested on twelfth day, when all had grown; fluid more or less stringy and not much clouded, rim and pellicle fallen; all negative. The presence of nitrate in each tube was then determined by obtaining the evanescent blue reaction with diphenylamine dissolved in sulphuric acid.

BEET.

Old tests by Miss Brown.—(1) 5 days, negative; (2) 33 days, reduced.

Tests in 1910 by Doctor Smith.—(3) 69-day-old culture by Miss Brown in her nitrate bouillon No. 600 (fluid strongly alkaline to litmus); on adding the nitrite reagents a copious blue, which does not shake out. (4) Plates poured and portions of 12 colonies transferred to the bouillon; tubes tested on the thirteenth day, when there were no strings or pellicle, but a white rim and a moderate amount of pinkish white precipitate; check and 6 inoculated tubes, negative; 6 tubes blue at bottom over the thick bacterial precipitate, but color shaking out of all.

COTTON.

Old tests by Miss Brown.—(1) 5 days, reduced; (2) 33 days, reduced.

Tests in 1910 by Doctor Smith.—(3) 69-day-old culture by Miss Brown in her nitrate bouillon No. 600 (fluid strongly alkaline)—copious blue color which does not shake out. (4) Plates poured and portions of 12 colonies transferred to as many tubes of nitrate bouillon. The tests were made at the end of 5 days when a good growth had taken place. Eleven tubes, negative. One shows a blue color at the bottom, but this shakes out. Presence of nitrate in these tubes was then determined by the diphenylamine sulphuric-acid test.

QUINCE.

Old tests by Miss Brown.—(1) 13 days, negative; (2) 5 days, reduced; (3) 33 days, trace of reduction, scarcely any growth.

POPLAR.

Tests in 1910 by Doctor Smith.—On July 25 three transfers were made from 3 subcultures to tubes of nitrate bouillon. The tests were made on the eighth day when there was a nearly clear fluid, a white pellicle covering the whole surface and not much precipitate, but some flocks and strings on one side next wall of tube. Result: Check and 3 cultures—all negative. Nitrate present in each tube. This was the organism called Flats poplar.

REMARKS.

A few of these contradictory results are to be explained on the hypothesis of contaminating organisms. When the blue color was merely local and shook out readily, the phenomenon would seem to be different in something more than degree from that ordinarily encountered. Whether nitrate reductions by bacteria are all alike and only a matter of degree, or whether there are two or more distinct mechanisms of reduction, is still an open question. Possibly one form is due to the direct action of sulphur compounds, while another depends on the activity of some enzyme.

The 1910 tests were made with 1 c. c. of opalescent boiled starch water (distilled water and starch prepared from potato in the laboratory); 1 c. c. of 1:250 fresh potassium iodide water, and 6 drops of 1:2 c. p. sulphuric-acid water. The checks were tested, the reagents were tested, and when results were negative the bouillon was also tested to see if nitrate was actually present. Frequently litmus tests were made and all of the cultures may be assumed to have been alkaline to litmus.

GROWTH IN BOUILLON OVER CHLOROFORM.

Inoculations from a 14-day peptone bouillon culture, examinations on the twenty-third day. All grew readily, except quince. Least growth in case of beet, chestnut, and newest daisy. To each tube containing 10 c. c. of +15 peptonized beef bouillon was added 5 c. c. of chloroform. The tubes were not agitated.

INVERSION OF CANE SUGAR.

The organisms were grown for 16 days in filtered river water containing 2 per cent Witte's peptone and 2 per cent c. p. cane sugar. On boiling with Fehling's solution (50 c. c. distilled water, 5 c. c. alkaline solution, and 5 c. c. $CuSO_4$ solution) the cultures fell into 3 groups as follows:

(1) Negative: Check tubes, sugar beet, new chestnut.

(2) Slight to moderate reduction. Apple hairy-root, new apple (slight), peach, grape.

(3) Copious reduction: Newest daisy, old daisy, new rose, hop, arbutus, alfalfa, Flats poplar, turnip.

All the organisms about which we felt any etiological certainty fell into class 2 or class 3.

TABLE X.—*Showing behavior [a] of crown-gall organisms in river water containing other stated ingredients, at room temperature.*

[Transfers from +15 peptone bouillon cultures.]

Organisms.	With 1 per cent Merk's asparagin added.		With 0.5 per cent each dextrose and glycocoll added (3-day-old culture).		With 0.5 per cent each dextrose and urea.		
	After 73 days (inoculated Mar. 5, 1910, 2-day-old culture).	Experiment repeated May 18, inoculating more copiously, (examined 43d day).	After 24 days.	After 2 months. [b]	Inoculated Apr. 23 (3-day beef bouillon). After 24 days.	Inoculated Apr. 23 (3-day beef bouillon). After 69 days.	After 73 days (inoculated Mar. 5, 2-day beef bouillon).
Daisy:							
Old strain	4	....	2	2	3	3	0
Newest	2. Thin fallen pellicle; does not break up easily.	....	2	2	2	2 (?)	[c] 2
Peach	4 or 5	....	2	2	3	3	4
Hop	4 to 5	....	0 (?)	2	0 (?)	0	0
Rose:							
Old	4 or 5	....	....	....	....	....	[d] 4
New	0	4	2	2	0 (?)	2 (?)	4
Apple:							
Old	0	0	0	0	0 (?)	0	0
New	3	....	....	....	....	....	0
Apple hairy-root	2	....	0	0	0	0	0
Alfalfa	0	2	0	0	2	2	0 (?)
Grape	0	3	0	0	2	2 (?)	2
Old chestnut	0	....	0 (?)	0	0	0	0 (?)
Arbutus	0	2	2	2	....	[e] 0	0
Beet	3	....	0	0	2 (old)	2 (old)	0 (old)
Cotton	4	....	0	0	[g] 0 (?)	[f] 4	4
Quince	0	0	[h] 0 (?)	0	0 (?)	0	0

[a] Explanation of figures indicating growth: 0 = No growth; 2 = slight; 3 = fairly good; 4 = good; 5 = heavy.
[b] Possibly no use of glycocoll by any of the strains.
[c] No increase in an additional 69 days.
[d] Turbid; cloudy on shaking.
[e] Forty-four days.
[f] Forty-four days, another tube more copiously inoculated.
[g] No growth later.
[h] Plug wet.

TABLE XI.—*Showing behavior of crown-gall organisms in river water containing 2 per cent Witte's peptone and 1 per cent Schering's c. p. glycerine.*

[Transfers from peptone bouillon culture 3 days old; records made at end of 27 days at 23° C.]

Group.	Organism.	Character of growth.
1	Old daisy*, peach*, hop†, cotton*, new rose*.	Heavy white rim and dense pellicle; scant clouding of fluid; moderate precipitate. On shaking, a dense mass of coarse fragments fills the fluid; 10 to 20 times as much growth as in group 2.
2	Newest daisy*, apple hairy-root*, alfalfa†.	Moderate white rim and thin pellicle; slight clouding; some precipitate.
3	Arbutus*, grape*, old apple, quince, beet (chestnut did not grow).	Scant white rim, thin pellicle or absent; fluid moderately cloudy; some precipitate; about same amount of growth as in group 2, but distributed differently.

Tubes were now tested for indol. Those marked with a star (*) gave distinct indol reaction without heating; those with a dagger (†), on heating. The others were negative. (See Table IX, p. 147.) The indol reactions were not as deep red as in case of *Bacillus coli.*

EXPERIMENTS WITH LITMUS-MILK CULTURES.

AUGUST 2-3, 1910.

Experiments to ascertain the behavior of the various strains of crown-gall organisms in milk gave the results shown in Table XII:

TABLE XII.—*Showing reactions of crown-gall organisms in sterile lavender-blue litmus milk at 30° C.*

Strain.	Time.	
	3 days.	7 days.
Newest daisy	Bluer than check; no whey	Much bluer; no whey; strong pellicle.
Old daisy	do	Litmus now dulled to a uniform plumbeous; no whey.
Salsify	do	Much bluer; no whey.
Flats poplar	do	Uniformly much bluer; no whey; strong pellicle.
Old chestnut	Unchanged	Uniformly bluer than check; no whey.
Turnip No. 2	Bluer than check; no whey	Much bluer; no whey.
Arbutus	Pinkish at top; paler blue below; no whey.	Rose purple at top; mauve below; not coagulated; no whey.
Cotton	Bluer than check; no whey	Uniform color verging on plumbeous; no whey; pellicle.
Hop	do	Uniform dull blue, tending toward plumbeous; no whey; strong pellicle.
Apple hairy-root	Unchanged	Unchanged or nearly so.
Newest rose	Paler blue than check; no whey.	Uniform blue, verging to plumbeous; no whey; heavy pellicle.
Quince	1 cm. purplish whey; dulled purple below; curd dissolving.	Litmus color gone; whey translucent; curd digested (nearly). The only trace of color is narrow pinkish rim.
Old rose	Bluer than check; no whey	Uniform color, verging on plumbeous; no whey; strong pellicle.
Alfalfa	Bluer than check; trace of whey on top.	Uniform deep blue; no whey; heavy pellicle.
Beet	Bluer than check; no whey	Much bluer; no whey.
Peach	do	Uniform plumbeous; no whey; strong pellicle.
Old apple	Purple red throughout and whey separated (this also on second day).	Whey nearly colorless; pale pinkish firm curd at bottom.
Willow	Bluer than check; no whey	Much bluer; no whey.
Grape	do	Do.
Turnip No. 1	do	Uniform dull blue; no whey.
Newport poplar No. 1.	do	Much bluer; no whey.
New chestnut	Unchanged; i. e., like check	Slightly bluer than check; no whey.
Parsnip	Bluer than check; no whey	Much bluer; no whey.
Newport poplar No. 2.	Slightly bluer than check; no whey.	Nearly color of check; red rim.

AUGUST 13, 1910.

The litmus-milk cultures now fall into three distinct groups, as follows (temperature 28° to 30° C.):

(*a*) *Litmus a uniform gray;* milk fluid, no separation of whey, moderate rim, heavy white pellicle, moderate white precipitate: Flats poplar, peach, old rose, cotton, hop, old daisy, turnip No. 1.

(*b*) *Litmus distinctly reddened*, most at top: Old apple, quince, arbutus, Newport poplar No. 2.

In the first two the casein has been thrown down, the bulk of the fluid being whey which, together with the clot, is now nearly colorless, i. e., only pinkish, with more evidence of solution of the curd in quince than in old apple. In the other two the milk is still fluid, i. e., no separation into curd and whey, and not yet much reduction.

(*c*) *Milk decidedly bluer than check, no reduction;* in some a slight amount of whey on top of the fluid milk, in others no separation: Alfalfa, old chestnut, newest daisy, beet, parsnip, newest rose, new chestnut, grape, willow, turnip No. 2, salsify, Newport poplar No. 1. This group might be split again as follows:

(*a′*) Narrow pinkish rims and moderate pellicle over a uniformly deep blue fluid, and a white precipitate: Old chestnut, new chestnut, parsnip.

(*b′*) The same, but with a pinkish-yellow precipitate and no pellicle: Beet.

(*c′*) Heavy whitish pellicle, moderate white precipitate, uniformly dulled blue fluid: New rose.

(*d′*) Very wide white rim (1 cm.), heavy pellicle, and scanty white precipitate: Alfalfa.

(*e′*) Uniform deep blue with blue rims: Newest daisy, grape, salsify, willow, Newport poplar No. 1, turnip No. 2.

AUGUST 19, 1910.

Group *a*.—No change except in the color of the milk, which is now a muddy tan color, with the exception of Flats poplar, which is still gray.

Group *b*.—No change.

Group *c*.—(*a′*) Parsnip—no change; old and new chestnut—barely a trace of pink in the rims; no other change. (*b′*) Beet—no change. (*c′*) New rose—no change. (*d′*) Alfalfa—no change. (*e′*) No change.

AUGUST 26, 1910.

Group *a*.—All (including Flats poplar—see August 19, above) are a dirty brown (approximately broccoli brown, Ridgway). Milk fluid, no separation of whey.

Group *b*.—Quince: clot is being dissolved. Only slightest trace of color except in the rim, which is pink. Old apple—no change. Newport poplar No. 2—casein is being thrown down; very little reduction. Arbutus—milk coagulated, no separation of whey; no reduction.

Group *c*.—(*a′*) Parsnip: no change (milk fluid, bluer than check; no separation of whey). New chestnut—no pink in the rim, otherwise unchanged. Milk fluid, bluer than check; no separation of whey. Old chestnut—milk fluid, no separation of whey; but reduction is taking place. Milk approximately Ridgway's lavender gray.

(*b′*) Beet—milk fluid, deep blue; no separation of whey. Pinkish yellow rim and precipitate.

(*c′*) New rose—like old chestnut in color, but slightly bluer; otherwise as on August 13.

(*d′*) Alfalfa—no change except that milk is paler blue than on August 13 and 19.

(*e′*) Salsify and grape have white pellicles and are much paler blue than the others in this group (reduction probably taking place slowly); milk fluid, no separation of whey. No change in the others (all deep blue) except that newest daisy has a pellicle (whitish). Milk fluid in all, and no separation of whey. Apple hairy-root is exactly like check. Probably no growth.

The Flats poplar was tested in milk with results very different from those Brizi obtained with his *Bacillus populi:* Three tubes of sterile white milk were inoculated copiously on July 28, 1910, and kept at 28° to 35° C. In 28 hours there was no visible change. In 4 days no separation of whey or change in appearance of the milk. In 9 days a copious white bacterial pellicle, but still no separation of the whey or curdling of the milk.[a]

SILICATE JELLY.

The behavior of all the crown-gall organisms on silicate jelly was much alike (one test only). There was a feeble to moderate white growth divisible into two groups about as follows:

(1) Smooth surface, growth rather scanty: Old daisy, newest daisy, grape (? growth), new chestnut, arbutus, old apple, new apple, apple hairy-root, beet, quince.

(2) Surface papillate-rugose but smooth on the margins, growth more abundant: Cotton, alfalfa, old rose, Turnip No. 1, hop, new rose, Flats poplar.

INOCULABLE AND CROSS-INOCULABLE.

Whether the different behavior of galls on various individuals of different hosts, sometimes forming soft, rapidly developing spongy excrescences and sometimes hard, slow-growing, slightly elevated tumors, or abnormal clusters of roots, as for example in the apple, is due principally to individual differences in rate of growth or juiciness of the particular tissues involved, to the particular tissue first infected, or to some other cause, must be left an unsettled question. The writers are inclined to think that there are several races of the gall-forming organisms varying more or less in amount of virulence and in adaptability to various hosts. Starting from soft gall of the peach, hard gall was produced on apple; and in the

[a] On potato cylinders inoculated at the same time and from the same culture, the color of the slime at the end of 28 hours was white like that of the potato substratum. In 4 days the slime was thin and dirty white. In 9 days the bacterial layer, which had not increased much, was a dirty white, and the fluid slightly brownish. The slime was not yellow and there was no marked action on the starch. At the end of a month when tested with alcohol iodine the potato cylinder gave a strong starch reaction, but the color was purple instead of deep blue (check).

same way from soft gall of daisy the hardest of hard gall on daisy. But inoculating with an organism plated from hard gall of the apple into actively growing soft daisy stem a series of galls were produced more resembling the original hard gall of the apple from which the colonies came than any typical soft gall of the daisy (Pl. III). On the other hand, as already detailed, starting with an organism from apple hairy-root and inoculating into young apple tree roots one developed galls while the others developed hairy-root. From one of the galls, however, on the tree which developed only galls, an organism was plated out which looked typical for what was inserted, and this when inoculated into healthy sugar beet produced both galls and hairy roots, indicating that crown-gall and hairy-root are only two forms of the same disease. Clustered roots also formed on one gall on Brassica. This hypothesis is further borne out both by the fact that the hairy-root clusters often originate in slow-growing hard galls and by the observation that rootlets frequently appear on peach galls in early stages of their development, but do not persist. The same phenomenon, transitory for the most part, occurs frequently or occasionally in some other galls, i. e., daisy, grape, clover, alfalfa.

Attempts at cross-inoculation have shown numerous differences (Tables II and III) the explanation of many of which must be sought in further experiments. Strains taken from some hosts, e. g., daisy, peach, hop, were inoculated into other plants with great ease. The strain obtained from the rose was inoculated into other plants with difficulty, but inasmuch as tumors were not readily produced on the rose itself by such inoculations it may be only that we were unfortunate in the selection of our rose bushes or of the colonies for our subcultures, getting slightly virulent strains. It is certain from our experiments on the daisy that a virulent strain may gradually lose its power to infect when kept for several years under laboratory conditions, and it is very probable that in nature some strains are feebly infectious and others actively infectious.

But we know in case of certain bacterial organisms cultivated in the laboratory that lessened virulence can be restored by certain procedures and we are not warranted in assuming that such restoration may not also take place in the fields.

DISCUSSION OF QUESTION OF SPECIES, VARIETIES, AND RACES OF THE CROWN-GALL ORGANISM.

Have we to do with one species or several? The answer is not at hand. Indeed, to those who have read thus far, it must be evident that much further time will be required to decide positively whether it is best to regard all crown-galls as due to variations of one polymorphous species, or whether they should be separated into two or more

species. Certainly there are not as many species as there are host plants, and the ease with which in many cases cross-inoculations take place points rather to one collective species. The monotonous morphology also points in the same direction, but the evidence is not all in. In this connection the reader is advised to make comparisons with the literature on root tubercles of Leguminosae.

The differences we have observed may be noted by consulting the tables and these seem to be real differences, e. g., slight differences in color or amount or texture of growth, ability or inability to grow in Cohn's solution, reactions in litmus milk, toleration of acids, etc. The difficulty is we do not know exactly what these things mean in the microbian economy, nor what weight to give them as differential characters. All told, the points of resemblance or agreement so far as we have studied the subject are much more numerous and salient than the differences, and for the present at least we prefer to leave the group undivided, merely indicating the various cultures for purposes of convenience by the name of the plant from which derived, as daisy, peach, poplar, etc.

Certain very practical questions arise here, viz, would it be advisable in nursery and orchard practice to follow one galled crop by another crop subject to galls, e. g., peaches by apples or pears, raspberries by grapes or quinces, or roses by almonds? Admitting frankly that we do not yet know the extent of artificial cross-inoculability, much less that of natural cross-inoculability, and that many more observations and experiments need to be made before we can be quite certain in particular cases to what extent the galls are naturally cross-inoculable, the grower who reads carefully the evidence detailed in this bulletin will probably hesitate to take the risk.

LOCAL REACTION OF THE INOCULATED PLANT.

YOUNG VERSUS OLD TISSUES.

In general old and hard, slow-growing tissues are not favorable to the development of this disease, whatever the host species concerned. Inoculations into such tissues frequently failed. Inoculations into dormant tissues usually failed, even though such tissues began to grow in the course of a few weeks. Mature tissues are not suited for inoculation experiments.

The most uniform success was had when the inoculations were made into young and rapidly growing parts. In such cases it is often possible to obtain 100 per cent of infections (see Table II). Apparently it is sufficient to introduce the bacteria into any actively dividing tissues of root or shoot—cambium, xylem, phloem, bark, pith, or mesophyll. Whether the structure of the tumor tissue in such cases

is dissimilar and whether the metastases partake of the nature of the original tumor are subjects requiring much further study.

In sensitive tissues the tumor reaction begins at once, and can be seen as a slight elevation about the needle pricks as early as the fourth or fifth day, and in the form of perfectly developed, small, fleshy growths a few days later (daisy, peach, etc.).

STRUCTURE AND GROWTH OF THE TUMOR.

The gross appearance of these tumors when they occur on cruciferous plants somewhat suggests the "finger and toe" attributed to *Plasmodiophora brassicae*. It is not a remote inference that all phenomena of this character on the roots of crucifers should be attributed to bacteria, particularly as no clear-cut inoculations with Plasmodiophora have ever been obtained. By this is meant inoculations which would clearly exclude the possible presence of pathogenic schizomycetes. But the chances are against such being the fact. We have not made enough experiments to be able to say positively that crown-gall bacteria never occur associated with the Plasmodiophora, but in opposition to this view, and favorable to the autonomy of the finger and toe disease, is the structure of its tumor, which shows very little hyperplasia and a great amount of hypertrophy, especially of the cells occupied by the spores of the Plasmodiophora. Moreover, the phloem is a favorite point of attack in finger and toe. Probably the correct view is that these are two distinct gall diseases of crucifers. Writers on malignant animal tumors are correct in asserting that the Plasmodiophora tumor is anatomically quite unlike the tumors with which they have to deal.

The anatomy of the crown-gall having proved a much easier subject than the etiology, there is a considerable body of literature respecting the structure of the galls, the details of which need not here occupy much space, since we contemplate a special paper on the subject. Those who wish to know more may refer to the following authors for structure of the tumors of the plants specified: Almond and peach (Toumey); rose (Scalia); sugar beet (Briem); raspberry (Wulff); poplar (Brizi). The contentions of these writers agree in the main, and the principal facts set forth by them are not contradicted by anything we have observed.

Some additional observations and inferences may here find place. In crown-gall we may assume either (1) a direct stimulus to growth or (2) an indirect one through the removal of some normal inhibition. Probably the first is the true explanation. The tumors appear to be able to arise from any meristematic tissue, i. e., from any cells of the organism which are able to divide. They are not subject to any physiological limitation. In a way, of course, the growth that

takes place in crown-galls is like that seen in the regeneration tissue of wounds, but that growth is governed by a physiological need and ceases with the repair of the wound, whereas the gall tissue proliferates indefinitely, passes beyond the control of the plant, and becomes a wasting disease. So far as the tissues themselves are concerned, the chief difference appears to lie in the different distribution of the various elements, the overplus of parenchyma, the weakening of the conductive tissues, the persistent prevalence of meristematic (embryonic) cells, and of immature forms generally, e. g., defective vascular bundles. Crown-galls vary greatly not only in virulence, but in their structure from species to species and also from individual to individual within the species, depending, apparently, on where the tumor takes its origin, i. e., whether it begins in pith or bark or wood or on the lamina of a leaf.

Sometimes the tumors are very woody and hard, their structure consisting chiefly of twisted and contorted lignified vascular bundles and woody fibers mingled with more or less parenchyma. At other times, and very often, the structure consists mostly of rapidly proliferating nests of parenchymatous tissue of a round-celled or spindle-celled type (Pl. XXXII), intermixed with which are vascular bundles (conductive tissues), more or less lignified, but twisted out of their normal shape, with walls abnormally thin, the total mass of the conductive tissue being less by far than that encountered in normal tissue, i. e., there is an enormous excess of the rapidly proliferating parenchyma and a corresponding reduction of conductive tissues.

The cells of the hyperplasia are often much smaller than the cells of the tissue in which it originates, e. g., inoculated tumors in cortical parenchyma of tobacco stem (Pl. XXIX). There is never any enormous stretching of individual cells such as we find a common phenomenon in the galls containing Plasmodiophora and in those formed by the nitrogen tubercle organism of Leguminosae. On the contrary, the stimulus to division is so active that the cells do not have time to attain their normal size. The mechanism of division is a subject for further research. In young, rapidly growing daisy tumors fixed for that purpose we did not find many karyokinetic figures, but in a rose gall numerous double nuclei were observed lying close together in undivided cells. Toumey observed this in almond. In our pure-culture inoculations where several needle punctures have been made close together sometimes only one gave rise to a tumor; sometimes all or nearly all of them developed independent tumors which fused into one mass as growth continued.

A study of sections of the earliest stage of tumor development might lead to interesting results respecting the cells first infected. This we propose to undertake. The tremendous proliferation prob-

ably begins in a single cell or in a few cells and perhaps from a special tissue, but whether the impulse to division must always come from within the infected cell, this impulse being transmitted only to its daughter cells, and so on, or may also be external, influencing neighboring groups of cells, remains to be determined.

When this double phenomenon appears, to wit, overproduction of parenchyma and corresponding reduction of vessels, and it occurs very often not only in the daisy, but also in the sugar beet, peach, hop, and many other plants, the tumors do not appear to be able to obtain as much water and nourishment as is required to carry them beyond a certain point in growth, and portions of the morbid tissues slough off, necrosis following growth in the course of a few months. It is seldom that the primary necrosis involves the entire tumor; some portion of it, generally the margin, remains alive and proliferates more or less extensively the same season or the following season, forming additional tumor tissue, which subsequently extends the open wound by additional necrosis. Where the woody fibers are more abundant this phenomenon does not occur, or takes place at a more remote date. In other cases the tumor regresses and no new one appears.

SUGGESTED RELATIONSHIP TO ANIMAL TUMORS.

The writer can not help feeling that the phenomena displayed in a rapidly proliferating tumor of the type figured and described in this bulletin shows a likeness to certain tumors occurring in the animal body, namely, to sarcomata. These plant tumors often grow very rapidly, and when the plant is a small one either destroy it within a few months or greatly injure it. They seem to be much more nearly related to sarcomas than they do to inflammatory processes, to which some of the animal pathologists with whom we have talked have been inclined to liken them.

Exclusive of the presence of leucocytes, which do not occur in plants, and of swelling, which even in animals is not the invariable accompaniment of an abscess (e. g., abscess in bones), we have in plants phenomena quite like abscesses in animals, but these phenomena are in no way like crown-galls. They consist of the formation in the stems or other parts of plants of more or less extensive cavities or chains of cavities filled with fluid, broken down portions of the tissues, and a greater or less number of the bacteria which have caused the disorganization, together usually with saprophytes of various kinds. Such phenomena occur in bacterial diseases of potato, maize, sugar cane, cabbage, pear, etc., but they are purely disorganizations (areas of softening), not *abnormal organization processes.* In the abscesses no new organs are formed. The most the plant is able to

accomplish under conditions favorable to it is the formation by cell division of a protective cork layer about the diseased area somewhat as the tissues of the animal body for the same purpose inclose tuberculous nodules or syphilitic gummata with a fibrous mass of connective tissue. According to the current medical classification of tumors, crown-galls belong with the infectious granulomata apart from the true tumors, but it is not likely that such classifications represent anything more than a temporary phase of progress in pathology, because they rest largely upon absence of knowledge. One by one as the causes of tuberculosis, lepra, syphilis, actinomycosis, etc., have been discovered these diseases have been removed by medical writers from the domain of tumors and classed as specific inflammations, but logically if parasites should be discovered all the remaining malignant growths would have to be removed, leaving nothing but the empty pigeon hole for tumors.

Unlike teratomas, these tumors do not have a restricted growth comparable to a defective normal growth. Teratomatous growths are frequent in plants, but quite unlike the cell development here in question. Neither are crown-galls to be regarded as degeneration processes. We have in plants certain disease phenomena, namely, œdemas, which seem to be more like degeneration processes in animals than are the growths here described. In œdema, which is believed to be nonparasitic, we have swelling from excess of water supply and more or less enlargement of parenchyma cells, but it does not usually pass beyond simple hypertrophy and does not involve such heterogeneous hyperplasiac tissue changes as are conspicuous in the crown-galls. Some of our gum diseases of unknown origin show somewhat similar degenerations, formation of internal cavities, with enlarged cells in the walls. In crown-galls no abscess cavities have been observed.

Cancers occur in a variety of animals, and no good reason has been advanced why they should not occur in plants. These tumors are morbid new tissue developments tending to weaken and destroy the plant, and their structure does not suggest galls due to insects. Insect galls are usually of quite specific structure and definitely restricted growth, whereas the crown-galls are of indefinite structure and indeterminate growth. As a working hypothesis, we may regard insect galls as due to a localized and fleeting stimulus of a chemical nature not unlike the more generalized and prolonged stimulus which leads to cell division in crown-galls. The determination of the immediate cause of cell division in the one would probably throw much light on the other.

Certain resemblances to malignant animal tumors may be pointed out in more detail.

In the crown-gall there is not only enormous proliferation of the parenchymatic tissues (often in nestlike masses), but there occur in the tumor also all the other tissues normal to the organs attacked, although usually the woody tissues—the conductive ones—are greatly distorted and reduced very much in volume. They are there, however, permeating the tumor in various directions. Some portions of them are seen on sections as small ligneous islands and others as more or less lignified short tubes; but these fragmentary appearances are due simply to the direction of the knife cut, and careful dissection of the part shows that the ligneous conductive tissues arise from the base of the tumor and twist and branch in various directions through it, becoming reduced to widely separated single vessels or pairs or small groups of vessels in the remoter portions. This fact should, perhaps, count for more, in our judgment, as to the analogies of these tumors than the appearance of the rapidly proliferating parenchyma cells, which, however, strongly suggest malignant tumor tissue of animals, as may be seen by referring to Plates XXVI to XXX. (See also Plates VIII and XXI.) Undoubtedly many of the supporting elements in crown-gall, perhaps all, grow out of the substratum along with the growth of the tumor (Pl. XXVI, lower figure, *Y*). In some instances in large tumors it would seem as though some of the vessels were produced in place from the tumor itself, but of this we are not certain.

Another suggestive likeness is the fact that in this disease, and likewise in the olive-tubercle, there are well marked metastases, that is, secondary tumors arising from within, at some distance from the primary tumor, as the result of migrations, but as yet in the crown-gall we do not know the mechanism of this migration, i. e., whether the bacteria move independently through the tissues, setting up irritations in more or less remote places, or whether the migration takes place only within special host cells. In many plants cells push through small pits in vessel walls, forming in the interior of the vessels numerous rounded growths known as thyloses. These often contain bacteria. If they should become dislodged, they might then fall or be carried upward in the direction of the water current to become, if still able to divide, the center of a new growth elsewhere, quite after the manner of malignant animal tumors, assuming metastases of the latter to originate always in this way. Observations of Hunger on the brown rot of tomato and of the senior writer on the same and on mulberry blight show that thyloses are developed in vessels as the result of bacterial infection. Usually, in thyloses the irritation is temporary and tumors are not developed, although in case of the roots of old cucurbits it is common to find woody vessels compactly filled with a pseudo tissue composed of thyloses.

Whether this suggested mechanism of distribution actually occurs in the crown-gall must be left for further study. Owing to the absence of visible channels of infection and to the difficulty of staining the schizomycete in situ, we do not know where nor to what extent it occurs in a dormant condition outside of the tumor proper; but in a very interesting and destructive fungous gall of West Indian lime trees, studied in the Laboratory of Plant Pathology for several years, Miss Florence Hedges has demonstrated that the fungus may grow through the stems to a distance of several feet from the primary tumor before an internal secondary tumor develops. The distance in one case was so great that the writer of this paragraph supposed it to be a second external infection until proof to the contrary was obtained by tracing the internal mycelium through the wood from the primary to the secondary tumor. Here the bulk of the abnormal growth consists of wood. A bulletin on this subject is in preparation.

It is probable that the parasite in its migrations from one part of the plant to another does not make free use either of the vessels or of the intercellular spaces, at least we have not been able to find it in them. Rather we think it is imprisoned within the specially stimulated and rapidly dividing cells and is by the growth of these cells carried along. The location of the visible metastasis would then depend on where the most favorable conditions for rapid growth developed. There would then be a slight chain of morbid cells all the way from the primary tumor to the secondary one. Further studies are necessary.

Plate XXX shows a photomicrograph made through a young metastatic tumor in the petiole of a daisy leaf. The whole interior is a mass of rapidly proliferating morbid tissue (parenchyma cells and distorted bundles), but it has not yet ruptured to the surface, the outermost tissues being the normal tissues of the leaf stalk. Later such a tumor would tear apart these tissues and appear on the surface. The primary tumor in this case was some months older and situated lower down on the stem. There are some indications in this section that tumor cells are growing between and wedging apart larger normal parenchyma cells (infiltration?), and this phenomenon may be seen conspicuously in the section shown in Plate XXVII.

A third likeness which seems to us of some importance is the fact that a certain degree of immunity can be induced in the plants by repeated inoculations. When several inoculations have led to the formation of successive galls, it is then usually impossible to induce galls in the affected daisies by inoculating again with pure cultures of the same strain which produced the initial tumors. One set of

successful inoculations does not suffice to produce this quasi immunity, but several are required. Even then it is possible by inoculating with a more virulent strain to induce tumors on these plants, although as far as our observation goes, the tumors are slower to appear and generally smaller and less vigorous in their growth than on check plants (p. 177). Spontaneous recovery from the disease is quite frequent.

A fourth likeness is the tendency of the disease to appear in callous or scar tissue, e. g., on pruned roots or at the junction of stock and graft. This appears to be rather more than mere presence of wounds in these places. The wounds must probably exist, but the softer, modified character of the new tissue appears to invite both wounds and infections, just as it also invites secondary fungous and bacterial infections.

A fifth resemblance consists in the marked tendency of the galls to return after excision.

Sixth, the fact frequently observed by us that on agar poured plates made from tumors, especially those of some age, certain colonies which look like those of the right organism and which behave properly when transferred to peptonized beef bouillon either do not produce the overgrowth when inoculated into susceptible plants, or yield only very slow-growing, feeble, soon stationary hyperplasias, requires explanation and may be mentioned here. At first these results were interpreted by us as meaning accidental presence in the tumors of organisms resembling *Bacterium tumefaciens* on agar, etc., but unlike it in other respects. More recently we have come to the conclusion, or rather formed the working hypothesis, that these perplexing colonies, or at least some of them, must be nonvirulent strains of the gall-producing organisms, not other species. We do not know what constitutes virulence, but we do know that on culture media many organisms gradually lose this property, *Bacterium tumefaciens* being one of them. The question then arises: Why should not virulence often disappear from organisms buried inside the tissue of tumors? And is not the fact that the tumor has ceased to be active and the host has gained the ascendency evidence of this? It is certainly conceivable that either by the juices of the host or through their own by-products the bacteria might be so acted upon as to lose power to infect other plants when cultivated out, and this without losing their common cultural characters. The same phenomenon is believed to occur in cultures of the organism causing root tubercles of Leguminosae.

It is believed by us that we have here the beginning of a solution of the cancer problem in men and animals, or at least a most instructive border-line field. The chief objection raised by animal pathologists with whom we have talked to considering these tumors in the

light of cancers is the fact that we know them to be produced by a specific organism, hence they are granulomata. If we did not know them to be so induced, then they would be willing enough to consider them as tumors. This is shown by the fact recently called to our attention, i. e., after these pages were prepared for the printer, that in the International Conference on Cancer at Paris in October, 1910, Professor Jensen (of mouse-tumor fame), not knowing of our researches, presented without hostile criticism a paper (Von echten Geschwülsten bei Pflanzen) on the crown-gall of the beet, in which he maintained it to be not only a true neoplasm, but a genuine tumor for which he predicted a rôle as important in cancer research as the mouse tumor itself has played. His exact words are:

Obwohl meine Untersuchungen nur noch einen vorläufigen Charakter haben, glaube ich doch, aussprechen zu dürfen, *dass wir beim "Wurzelkropf" nicht nur mit einem echten Neoplasmus sondern gar mit einem Tumor zu schaffen haben, der in gewissen Beziehungen Aehnlichkeit mit den malignen Geschwülsten der Tiere darbietet; ja, ich bin geneigt zu glauben, dass er in der Geschwulstforschung eine ähnliche Rolle spielen können wird, wie jetzt die Mäusecarcinome.*—Page 248.

And again, on page 254:

Wir haben also in dem sog. Wurzelkropf *eine Geschwulstbildung* vor uns, *die auf einer andauernden, abnormen Proliferationsfähigkeit gewisser Zellen zu beruhen scheint, und die nicht nur dadurch, sondern auch durch ihre Beeinflussung des Wachstums der Rübe, ihre Fähigkeit zu rezidivieren und sich transplantieren zu lassen, so wie durch die abnormen chemischen Verhältnisse der Zellen so sehr an die malignen Tumoren der Tiere erinnert, dass ein näheres Studium der biologischen Verhältnisse der Geschwulst unzweifelhaft wohl angebracht wäre.*

The animal pathologists have not come to any agreement as to what is the cause of sarcoma, carcinoma, and similar tumors, some holding them to be due to organisms, either known or suspected, while others, now the majority, maintain that inasmuch as inoculations of certain ground cancerous tissues have not led to any infections and inoculations of uninjured tumor tissues of the same sort have led to numerous and repeated infections, therefore the disease can be transmitted only by the living proliferating cancer cells, e. g., experimentally by grafting. As clear a statement of this view as any, perhaps, is that given by Dürck:

The essential difference between infectious growths and genuine tumors is that when the former are reproduced by metastasis the parasite itself is conveyed in the blood and incites at the metastatic site new formation of tissue similar to that of the parent growth, whereas in the case of genuine tumors metastasis takes place by the transplantation of a part of the parent tumor, which then begins to proliferate independently at the new site.

But we are totally in the dark as to what originates cancer cells or causes them to proliferate.

It has been known for a considerable period, i. e., since 1900, that crown-galls could be inoculated into healthy plants by means of

pieces of tissue and that tissues so inserted would grow into a new tumor, and in that period we were in precisely the same condition as the animal pathologists of to-day, who reproduce mouse tumors and similar malignant growths by introducing pieces of the diseased tissue under the skin of healthy animals, but can not explain the reason why. We did not then know that such plant tumors were due to a specific organism, and a good many of us were very skeptical as to the existence of a parasite, because after repeated careful searches by a good many people no such organism had been demonstrated in situ by means of the microscope, and the things which had been plated out of such plant tumors and tested on healthy plants had produced in them nothing comparable to the growth from which they had been taken. Enough such experiments have been made and by a sufficient number of persons to show that unless one knows just how to set to work it is not at all easy to obtain the pathogenic organism from crown-galls. As stated in the beginning of this bulletin, we boggled away at the problem a couple of years before we were certain by isolations and inoculations that we had in a particular micro-organism the specific cause of the disease. Our troubles were of various sorts. First of all the tumor tissue when it has reached any considerable age is rapidly invaded by secondary organisms, i. e., saprophytes, and on the plates these are the ones that we commonly obtained. Isolation is also complicated by the fact that the organism which is the cause of the disease is a rather sensitive one, i. e., frequently is killed out quickly in the struggle with saprophytes. The problem was further complicated by the fact that on standard +15 nutrient agar, which was our common substance for poured-plate isolations, the initial growth of the bacteria taken directly from the interior of the tumor and distributed in the agar plates is extremely slow, so that often colonies visible to the naked eye are not seen before the fifth or sixth day, and sometimes not until the tenth or twelfth day or later. In other words, the saprophytes would come up quickly and be studied and the overgrown plates discarded before the right organism would appear at all.

The writers also believe that the organism in the tumors either multiplies very slowly or if growing at an ordinary rate is killed off rather rapidly by the chemical reactions of the plant itself, or by the by-products of its own growth. We have been led to this hypothesis by several facts. First, it is not easy to obtain stained preparations of the tumors in which the bacteria can be demonstrated. After six years we have to show not a single good preparation. We get numerous granules of the size of bacteria, and some of them of the general outline, but so far with few exceptions none which take a sharp stain leaving well-defined walls such as one looks for in order to be reasonably certain that he has bacteria in his preparation and not

something else, e. g., cell detritus. The fact that the organism as it occurs in the gall comes up slowly on agar poured plates, but grows as promptly as other bacteria in the same medium when transferred from cultures, may be coupled with the fact that irregularly shaped involution forms are common in this species when it is exposed to certain unfavorable conditions, e. g., cold or sodium chloride. If such involution forms were the common form also in the plant, it might explain many of our failures. Such club-shaped and branched forms occur abundantly in some of the nitrogen root nodules of Leguminosae.

If this were true, namely, that there is a pretty nearly even balance between the growth of the bacteria in the tumor tissue and the destruction of the same, then we might have the tumor rapidly proliferating as the result of the stimulus of enzymes, toxins, acids, alkalies, or other substances, dissolved out of the dead bacteria, and not in the tumor at any given time very many bacteria demonstrable by means of stains, because inactive and partially disorganized bacteria are proverbially difficult to stain.[a] Might not some phenomena of the kind mentioned be present in malignant animal tumors and thus complicate the determination of their etiology? We know in case of the crown-galls, even when we can not stain the bacteria in the tissues, that they are there, because by selecting small tumors no part of which has yet passed into a necrotic condition we can obtain therefrom cultures of the gall-producing organism, and have done it over and over again. All that it is necessary to do is to scrape thoroughly and wash the unfissured surface of the gall, then soak it in some germicide long enough to sterilize the surface (an hour or less in 1:1,000 mercuric chloride water usually suffices), dry it, and dig into the depths of the tumor (or in case of hard tumors into superficial rapid-growing portions) with sterile instruments, remove and crush some of this interior portion in sterile bouillon, and make poured plates in +15 nutrient agar. We are further led to the belief that living bacteria are not numerous in the galls by the fact that even when the melted cooled agar for plates is inoculated rather copiously, what one would ordinarily call very copiously (say, 1,000 or 10,000 or even 100,000 times too much) if he were dealing with other diseases, the colonies developing on the plates (which under the conditions mentioned are sometimes absolutely free from intruders) are not very numerous. Third, even this procedure will frequently fail to yield any colonies, unless one also takes the added precaution of allowing the living bacteria present in the partially

[a] Since this was written we have obtained numerous involution forms in agar and bouillon by adding weak acetic acid. These facts, coupled with the knowledge derived from the flask analyses, viz, that acetic acid is formed from sugar by this organism, makes it very probable that both acetic acid and involution forms occur in the tumor.

crushed tissue ample time to diffuse out into the bouillon by letting it stand for half an hour or an hour before the plates are made. This is additional proof that the number of viable and stainable bacteria in any given portion of the tumor tissue is rather small, so as to be hard to find, unless it be that the bacteria are abundant but intracellular to such an extent, i. e., so intimately mixed with the protoplasm, that they do not readily diffuse out of the partially crushed tissues. Certainly there is nothing in the crown-gall comparable to the phenomenon seen in the nitrogen tubercles of legumes where the hypertrophied cells become gorged with bacteria, easily seen as such whether stained or unstained. With good technique the right organism can be obtained by means of poured plates from almost any rapidly proliferating part of the crown-gall tumor, but often not at all if one tries from older portions of the growth, i. e., those more remote from the active centers of growth.

The isolations are also sometimes complicated by the fact that of two colonies on an agar poured plate looking just alike, one may be able to cause the disease and the other destitute of pathogenic properties.

The difficulties we have encountered in determining the etiology of these tumors make it only reasonable to suppose that similar difficulties would be encountered in isolating the parasites of animal tumors, admitting for the time being that they are due to organisms. This is also suggested by the past difficulties encountered in determining the cause of tuberculosis, lepra, and syphilis. A few suggestions may be offered for the consideration of pathologists who believe malignant animal tumors to be of parasitic origin, but have not been able to demonstrate the suspected parasite.

(1) All present cellular theories of cancer origin are incompetent to explain how such cells originate, i. e., become cancerous, or why they multiply; and in the light of the facts here presented we would suggest that renewed search be made for a parasitic organism or virus either independent of specific cells or confined to them and using them as a means of dissemination. It would seem that the initial cancer cell or cells in an organism must result from the action of a foreign organism or virus, whatever may be thought of the process of abnormal growth once established.

(2) If we may assume the suspected parasite to be present in the tissues in an active state in very small numbers only, owing to the nearly balanced struggle of the host against the invading organism, and the rapid destruction also of the causative organism in many parts of the tumor, owing to the invasion of saprophytes, then one might well have failed to produce the disease by injection of small quantities of ground-up cells without this being conclusive evidence

of the nonparasitic origin of the tumor, either no parasites being introduced or so few and these so reduced in vitality by long presence in the tumor or by exposure to the juices of the crushed cells, which we may suppose to be more or less germicidal, that they are overcome and destroyed by the normal activities of the body. It is possible also that there may be some special mechanism of infection. Here might also be pointed out that most of this evidence has been derived from mouse tumors, and that we are under no obligation to consider all malignant tumors as etiologically identical.

(3) A parasite might be present and not isolated because unable to grow on the media commonly offered to it, as in the case of syphilis and yaws. The most striking evidence of this nature the senior writer has had brought to his attention was the failure of a streptococcus associated with endocarditis to grow on media obtained from one of the best human pathological laboratories in the country, but which grew readily in slightly different media. The growth of the organism, as was afterwards determined by him, was inhibited by the presence of too much sodium hydroxide in the bouillon. This particular organism he also observed to be very sensitive to sodium chloride, so that a slight excess of sodium chloride in the agar or bouillon would also inhibit growth. Had only one bouillon or agar been used the experiment would have failed. This organism was isolated in +15 agar and bouillon, but would not grow in zero bouillon or agar (October, 1906).

In the light of these facts there can be little doubt that many of the blood tests in arthritis and endocarditis which have been described as negative by various physicians and surgeons are to be regarded as failures due to the use of improper culture media, rather than as proof of absence of organisms in the blood or other fluid tested. Why not failures of this kind also in other fields of animal pathology? In recent years but few serious attempts appear to have been made to isolate a parasite from malignant animal tumors.

The variety of difficulties encountered in obtaining cultures of the organisms causing tuberculosis, lepra, syphilis, rabies, etc., should also be considered; e. g., pathologists have been satisfied for a long time as to the cause of leprosy, being able to stain a certain acid-fast organism within the cells, but not until very recently has it been possible to grow it in pure culture and with subcultures therefrom reproduce the disease in mice (Duval: Jour. Exp. Med., 1910, Vol. XII, pp. 649 to 665).

(4) Failure to demonstrate the supposed parasite in stained sections might be due either to its scarcity, to its indifference to stains, to its lack of power to retain them during the washing, or to the fact that it may occur in the tumor in some very minute or unusual form,

e. g., in involution forms. In this connection see a paper by S. B. Wolbach and Tadasu Saiki on the presence of bacteria in normal livers, demonstrable by cultural methods but not by stains (The Journal of Medical Research, Boston, September, 1909, p. 274).

(5) The likeness of crown-gall to animal tumors might be thought at first sight to be lessened, owing to the fact that plants of many sorts can be made to take the disease by means of grafting or pure culture inoculation, whereas animal tumors are supposed to be very restricted in cross-inoculability. One reason for this difference may lie in the greater simplicity of plant structures, plants being much less highly specialized than vertebrate animals. It is possible also that the doctrine of non-cross-inoculability of animal tumors may be a sweeping generalization based on insufficient evidence. Recently Van Dungerm states that he has successfully inoculated sarcoma of the rabbit into the hare; and Sticker claims to have produced dog tumors in the fox.

(6) The most difficult thing to explain on any parasitic theory is the character of the metastases in cancer. These are so characteristic, and so like the tissues of the original tumor that from an examination of sections of the secondary tumor it is often possible to determine where the unseen primary tumor is located, whether, e. g., in the stomach or the ovaries. This, however, does not seem to be an insuperable objection. Vide Mühlman, Ueber Bindegewebsbildung, Stromabildung und Geschwulstbildung—Die Blastocyten Theorie (Archiv. f. Entwickelungsmechanik, 28 Bd., pp. 210–259).

It is not yet beyond dispute that a cell mother of one kind can never give rise to a cell of another kind when a changed stimulus is applied. Adami and several others maintain that particular animal cells forming a normal part of tissues, i. e., not in juxtaposition with the proliferating mass of morbid tissue, may become cancer cells.

METASTASES.

It had been noticed during the early part of our work with the gall organisms that when a daisy plant, never before affected, was inoculated and galls were produced, the disease did not confine itself to the inoculated part or its immediate neighborhood, but made its way to other parts of the plant. This was shown by the marked tendency of galls to form on leaves or parts of the stem other than that part on which galls developed as the result of our inoculations.

Some of these galls may have been due to accidental surface infections, but it seemed that all of them could not be ascribed to local surface infections for several reasons, i. e., because the check plants in the same house remained remarkably free from infection, because the hothouse was quite free from small animals likely to cause

wounds, and because some of these galls were observed to arise from the deeper tissues and to push up the sound superficial tissues (Plate XXX) several days in advance of any actual rupture of the latter.

When the first cuttings were made from the first galled plants, notes were kept of the behavior of these cuttings, and, of 33 made, 18 developed galls within six weeks. Some of the galls were underground on the base of the cutting, some were at the surface of the earth, and some were on the upper part of the stem.

Finally, experimental inoculations into the leaf-traces under the point of insertion of the leaf, caused, first, the appearance of galls on the stem where the needle entered, and subsequently at a distance, internal galls. These internal galls appeared along the line of the punctured leaf-traces in the petiole and on the midrib of the leaf, several centimeters from the primary galls, and gradually ruptured to the surface. The plants selected were sound and there could be no question of the secondary galls having originated *from within, and as a result of some stimulus due to the primary gall*, both because they appeared exactly where it was reasoned out in advance that they should appear, and because they were watched through all stages from the first slight elevation of some portion of the sound midrib until through stress of internal tensions it finally split open, showing the tumor tissue in the bottom of the cleft, which tissue gradually increased in size until it projected far beyond the borders of the crevice as a typical gall.

These growths developing from within outward must be due to migrations or growths from the primary tumor (of bacteria certainly, of host cells inclosing the bacteria probably), but we have not been able to demonstrate the channel of migration either in unstained or stained sections. Cuts made at various points between the primary and the secondary gall yielded nothing to the microscope, nor did we obtain bacterial colonies on agar poured plates made from such tissues, but this is not surprising considering the relative scarcity of the bacteria in the galls themselves. In the olive tubercle, which is superficially like the crown-gall, there are abscess cavities filled with the parasitic bacteria, and a distinct channel of infection can be traced from the primary tumor to the secondary (metastatic) tumors. This occurs in the wood following the path of certain spiral vessels situated at the inner border of the xylem next to the pith. Here distinct lesions occur. On cross section the path of migration in the stem can be seen with the naked eye in the form of small brown dots (lines on longitudinal section) from which under very favorable circumstances a white bacterial slime may be seen to ooze in minute quantity. Under the microscope this browned area is seen to be occupied by bacteria. The vessels and

surrounding tissues in which these bacteria lie are not only stained, but otherwise disorganized. Nothing of this sort occurs in the crown-gall. The subject is still under consideration.

The anatomy of one of these metastatic tumors in a very early stage of development is shown on Plate XXX. All the central portion of the section is occupied by the incipient tumor. The white lines on the margin mark off the extent of uninfected tissue. The tumor had not yet ruptured to the surface, but would have done so in course of a few days, on the upper part of the section, where the abnormal tissue is nearest to the surface.

CHEMICAL CHANGES.

EXCESS OF OXYDIZING ENZYMES IN THE GALL TISSUE.

The oxydizing power of extracts from crown-galls is greater than that of extracts from sound tissues. Toumey showed this for almonds. It was also shown by Miss Marian L. Shorey in some determinations made for the senior writer in 1908, using sugar beets. These beets had been inoculated for some months with the daisy organism and bore moderate-sized tumors. The black powder isolated and purified by repeated precipitations with alcohol was introduced by knife wounds into the crown of many growing sugar beets, but no tumors resulted. This excessive production of colorless substances oxydizing readily to dark compounds on exposure to the air is to be regarded as a host reaction, and is perhaps due to an increase in the oxidase content.

In 1909 (Blätter f. Zückerrübenbau, XVI Jahrg., Nr. 6) Reinelt mentions that Bartos had observed the gall substance in sugar beet to be somewhat darker than the rest of the beet, and says that he himself observed that when beets are placed in absolute alcohol or vapor of alcohol this difference in color becomes more pronounced, the gall becoming very dark, whereas the body of the beet is but little stained. In some tests made in 1910 the senior writer observed the same difference *but only so far as regards the outer protected surface.* When the galled beets were thrown into alcohol the galled parts turned dark almost immediately, while the smooth part of the root (protected by a normal bark) remained white. No such contrast was observed, however, when the same beets were sliced so that the alcohol had an equal opportunity to act on all the tissues. These were the beets which served for the illustrations shown on Plate XXII.

OTHER CHANGES IN THE TISSUES.

The chemical analyses by Strohmer and Stift (Österr. Ungar. Zeits. f. Zuckerind. und Landw., II Heft, Wien, 1892) show in the

crown-galls of the sugar beet, as compared with the unaffected parts of the same roots, slightly more water, considerably less cane sugar, the presence of invert sugar, double the quantity of ash, and in all but one instance more than double the amount of raw protein. Six analyses were made and the calculations are expressed in per cents of fresh substance. They all agree, except that two blanks occur in the invert-sugar line, and two in the raw-protein line.

The same facts respecting cane sugar, invert sugar, pure ash, and raw protein are shown still more strikingly in a table where the amounts are calculated in 100 parts of the dry substance. No invert sugar was found in the normal parts of the roots, but 0.91 to 1.52 per cent in the galls. An average of the six analyses shows that the dry substance of the galls contained 50 per cent cane sugar as against 61 per cent in the normal parts of the roots. The average per cent of ash in the roots examined was 2.78 and in the galls 6.05. The average per cent of raw protein in the roots was 4.09 and in the galls 9.80.

ANALYSIS OF FLASK CULTURES OF BACTERIUM TUMEFACIENS.

Analyses of flask cultures of the daisy organism after some months' growth in 750 cc. filtered river water containing 35 grams c. p. calcium carbonate, 14 grams Witte's white peptonum siccum, and 35 grams Merck's c. p. dextrose were made for us by Dr. Carl L. Alsberg, with the following results:

Received July 26, from Doctor Smith, five flasks of the culture, labeled, "Daisy (newest strain)." The reaction of the culture medium [inoculated March 29, 1910] was distinctly alkaline; the bottom of the flask contained much calcium carbonate, which was filtered off. The filtrate was alkaline. A small portion, when acidified with acetic acid and treated with ammonium oxalate, gave a heavy precipitate of calcium oxalate, showing that a considerable amount of the calcium carbonate had been dissolved. The solution reduced Fehling's solution powerfully, showing the presence either of aldehyde or of sugar. Subsequent investigations showed the absence of aldehyde, so that this reduction must be attributed to sugar. Other flasks of the same lot, the analysis of which was taken one or two months later, still showed a large quantity of sugar present. The filtrate, which was alkaline, was preserved and examined. It did not reduce ammonium silver nitrate solution, and therefore can not have contained any aldehyde. It gave a powerful reaction with potassium iodide, resulting in the formation of considerable iodoform. Hence, the main constituent of the distillate was ethyl alcohol. The residue in the distilling flask was now acidified with sulphuric acid, and the distillation repeated. The distillate proved to be very acid, and had an odor resembling acetic acid. It was made ammoniacal and concentrated to a small bulk. The neutral solution resulting was treated with silver nitrate, yielding a crystalline precipitate. This was recrystallized in hot water, yielding large white needles; 0.3969 gram of this silver salt yields 0.2559 gram of silver, or 64.46 per cent of silver. Silver acetate contains theoretically 64.67 per cent of silver; hence the volatile acid can not be anything else than acetic acid.

The results obtained with this single culture flask were exactly duplicated with two other flasks.

Another portion of the culture was acidified and shaken out with ether. The ether was driven off, leaving a yellow, oily residue which contained a very small quantity of colorless, radiating, short prisms. These were insoluble in water, and had every appearance of fat. It was attempted to discover whether the residue contained any other acids, by preparing the barium salts and fractionating them by means of abstraction with absolute alcohol. No lactic or succinic acid could be detected. The residue from the ether seemed to consist mainly of a little fat and some fatty acids.

The calcium carbonate, which remained in the flasks, was removed from the cultures by filtration in hydrochloric acid and extracted with ether, and no acids passed into the ether extract, so that this precipitate does not seem to have contained anything besides the calcium carbonate.

Summary: A considerable quantity of acetic acid and ethyl alcohol was identified in the culture medium. No other fermentation acid could be detected. There seemed to be present a small amount of fat or fatty acids.

THE STIMULUS TO GROWTH.

All plant tumors are not due to the same parasite, but all the hyperplasias are due probably to the same chemical substance or to closely related substances, whatever the organism may be that produces these growths. This substance, which we shall eventually isolate, is probably a by-product of the growth of the intruding organism, possibly a complex colloid, or perhaps only some comparatively simple substance acting continuously in minute quantities. It is our hope finally to cause the crown-gall with specific products of the bacterial growth freed from the living organisms and from extraneous substances, and we have under way already certain experiments of this sort, but they are not yet ready to be reported on.

As a first working hypothesis we have assumed some salt of acetic acid, possibly ammonium acetate, to be the cause of the stimulus, either, (1) as the primary source of the irritation, or, (2) as the liberator of such an irritant from the protoplasm of the bacteria through its killing action on their membranes, which would render them permeable.

PHYSICAL CHANGES—EARLY DECAY.

The physical changes in the tumors are such as would naturally occur in any rapidly proliferating parenchyma imperfectly provided with conductive tissues. It would seem that beyond a certain point the soft tissues can not be supplied with water and food, and decay sets in with more or less sloughing of the tumor and the appearance of open wounds. The harder and more slow growing the gall the later this appears. A variety of saprophytic bacteria and fungi take part in disintegrating the overgrown tissues. Among these saprophytic bacteria there are several white forms closely resembling the gall organism as grown on agar poured plates, dendritic white forms, green fluorescent species, yellow species, orange species, pink species, etc.

The nonpathogenic white forms generally develop on agar plates somewhat whiter or creamier or denser colonies than the gall organism. They look more like the latter in early stages of growth than after some days. But some resemble it so closely on agar that cultures on other media are required. From old galls it is often difficult to isolate the parasite, the tissues swarm with such a mass of secondary and tertiary forms. So true is this that from such parts it is scarcely worth while to attempt isolations. These are best made from the youngest growing parts.

Their fleshy nature also tempts parasitic fungi and bacteria, mites, nematodes, and a variety of insects.

When the tumors are very fleshy decay sets in earlier than when they are woody.

EFFECTS OF THE DISEASE ON THE TISSUES NOT DIRECTLY INVOLVED.

PHYSICAL EFFECTS.

The necrosis of gall tissues already mentioned affords opportunity for the entrance of rain water and many sorts of insects, bacteria, and fungi, which bring about more or less destruction of supporting tissues not involved in the original tumor. In this way the pear-blight bacillus and facultative wood and bark parasites of various sorts may enter, causing serious stem and root injuries. If the plant is an orchard tree it may be weakened by this decay of the wood so as to be easily broken off by animals or blown over by the wind. This often occurs in the peach and almond; rarely in the apple. Plate XXXI shows the bacterial apple blight (*Bacillus amylovorus*) originating in a hard gall.

PHYSIOLOGICAL EFFECTS.

The immediate and remote physiological effects of these tumors vary from species to species and also within the species and are generally less pronounced and certainly less speedy than we might expect from their size and vigor of growth. The plant, however, is less specialized than the higher animals, especially by absence of a nervous system, and in this connection it might be interesting to speculate on what would be the outcome of malignant animal tumors if the depressing influence of pain were removed and the consequent greater or less disturbance of all the functions of the body.

In many instances the tree shows no material injury even after a series of years. This is especially true of the apple, according to Hedgcock, Stewart, and others. In other cases, and this is true even of the apple, the attacked tree is dwarfed in comparison with its unattacked fellows. Peaches and almonds show this dwarfing to

a greater extent than apples, and roses in hothouse culture are still more conspicuous examples of it. Unfruitfulness has also been observed in the last three species and in the grape. A large part of this phenomenon is perhaps attributable to simple abstraction of food and water. In case of the daisy this often proceeds to such an extent that individual branches projecting beyond well-developed galls present a starved appearance and die prematurely.

This disease never induces premature development of blossoms and fruit so far as observed, but on the contrary retards development—rose, daisy, apple.

It is a difficult matter to determine whether the substances elaborated in the tumors by the parasite or by the saprophytes which follow it are absorbed and act as slow poisons on the remoter tissues, but there is some warrant in the appearance of the plants for this assumption.

Death of galled cuttings may occur within a few months, but ordinarily on well-rooted plants it either does not occur at all—i. e., the the plant outgrows the disease—or it occurs only after a lingering illness of many months or several years, and then frequently as the result of secondary infections due to other organisms.

In many of our inoculated daisies we have observed what we have interpreted as increased resistance due to the long-continued growth of tumors on the plants, and consequently there would appear to be reactions set up in the plant which are possibly comparable with some of those observed in the animal body. We do not yet know to what substance this increased resistance is attributable. The subject is dealt with more fully in the following chapter.

EXPERIMENTS SHOWING INCREASED RESISTANCE OF THE HOST DUE TO REPEATED INOCULATIONS AND ALSO DECREASED VIRULENCE OF THE BACTERIA.

While the work with the different gall organisms was being carried on extensively, a group of plants of the Queen Alexandra daisy or progeny of the same was used constantly for inoculating, and the diminishing size of the galls that formed in comparison with those of the first inoculations and also the longer period of time required for their formation drew attention to the fact that either the organisms used were less virulent than when they were first isolated or else that a change was taking place in the plants themselves. To determine which hypothesis was the correct one fresh daisy galls were taken and the organism plated out to get a strain which had not become attenuated through repeated transfers on culture media. The new strain was inoculated into cuttings made from galled plants which themselves had been cuttings from previous galled ones.

The results of the inoculations seemed to indicate that the change must be in the plant itself, for the galls that formed from the presence of this newly isolated organism were also slow growing and did not reach half the size of those galls produced when the first daisy plants were inoculated.

The idea then began to take shape that this failure of the organism to form a gall of the usual size when inoculated into the most favorable growing daisy tissue might be due to some substance developed in the plant for protective purposes, and experiments were planned to determine if daisy plants could be made immune to this disease through repeated inoculations into the same plant or into rooted cuttings made therefrom.

In the following tests the plants used were taken at their most favorable age—that is, they were inoculated when the tissue was young and tender, so that the organism would have the best possible opportunity to produce the disease. Because cuttings did not grow well in the winter months the work was confined generally to the spring and summer.

(1) In March, 1907, a dozen daisy plants of the Queen Alexandra variety were inoculated with the daisy gall organism. These plants had never been known to have galls and had not been inoculated before. In two months' time good-sized galls had formed at all the points of inoculation.

(2) Cuttings (first set) were made from the preceding plants in May, 1907. The cuttings were growing well in July and then a second series of inoculations were made on them. A dozen plants were used this time. Galls formed which were as large as those of the first series.

(3) In November, 1907, cuttings (second set) were made from the plants of the second series, but they did not grow well at first and it was decided to wait until growth had started up well in the spring before further work was done with them. The inoculations (third inoculations) were made in April, 1908. Galls formed at each inoculated place, but they were much smaller and grew very slowly. In August they were less than half the size of the galls of the first series.

(4) Twenty-five cuttings (third set) were made from these diseased plants on August 17, and inoculations (fourth series of inoculations) were made November 18, 1908, on a dozen plants two and three shoots each. In the meantime a new strain of the Queen Alexandra daisy was purchased from a florist and the virulence of the organism checked up on these new plants which had never been affected with the gall. Large galls formed on the new daisies in a month, but there were none on the third set of cuttings. This was the fourth time that strain had been inoculated.

At the end of a month (December 22, 1908), as there was no trace of a gall starting to form, the same 12 plants were inoculated again (fifth series of inoculations) further to test the case. These plants were watched carefully but no galls formed. In a few cases the tissue at the points of inoculation was raised a little as though the presence of the organism had had some little effect. As galls formed at every point of inoculation on the check plants the organism used for the inoculations was proved to be all right. However, four months after this last inoculation of the third set of cuttings, the plants were examined again, and a gall was found on the root of one of them and one on the stem of another where a cutting had been taken.

(5) In March, 1909, cuttings (the fourth set) were made from the plants which seemed to be immune, and on May 20 they were inoculated as follows (sixth series of inoculations), some with the daisy organism which had been used through the entire test (strain B), some with the peach-gall organism, and some with a daisy organism recently plated from a gall and proved up by other inoculations. Six to 8 shoots on each of 6 plants were inoculated with the old-daisy organism; 4 plants including a like number of shoots on each were inoculated with cultures of the crown-gall of peach organism; and 6 plants with cultures of the daisy-gall organism recently plated out. In all there were over a hundred inoculations, i. e., groups of punctures.

There were no daisy plants available for controls, so young sugar-beet plants about 6 inches tall were inoculated at the crown with the same cultures. Two beets were inoculated with cultures of the old daisy, 2 with the new daisy, and 2 with the crown-gall of peach organism. Sugar beets were used because they had been found to take the gall very readily.

On June 18, 1909, there was not a trace of gall formation on any of the daisy plants inoculated May 20. The checks of the peach gall and of the old daisy (both on the sugar beets) had good-sized galls, but those beets inoculated with the new daisy had none. These plants, however, were in a shady place and had not made much growth since the time of inoculation. The galls on the 4 sugar beets were accounted sufficient proof that 2 of the 3 strains were able to produce galls in susceptible plants.

The same day (June 18, 1909) some of the same daisy plants were inoculated again with the crown-gall of peach organism, 16 groups of punctures being made (seventh inoculation). The plants were growing very well. Five young sugar beets were inoculated at the crown with the same cultures as checks on the daisies.

On July 6, 1909, the plants were examined and no galls were found on the daisies; 2 of the 5 sugar beets had small galls which bade fair to increase in size as the beets grew.

This last set of cuttings (fourth set) in which two sets of inoculations had been made already was subjected to one more test. A fresh strain of the peach-gall organism which had been isolated in April, 1909, from some trees grown in Virginia was used for these inoculations. This organism was selected because it had produced galls very rapidly on a daisy plant which had never been affected with this disease. In July, 1909, 43 inoculations (eighth series of inoculations) were made. Five young sugar beets were inoculated at the crown with the same cultures used on the daisies. On August 30 the last inoculations of the daisy were examined and no trace of a gall was found on any. Of the 5 sugar beets only 1 had a gall; the beets had grown scarcely at all since they were inoculated, so they were repotted and left to develop. October 4: These beets never grew to any extent, but 1 other bore a tiny gall.

On September 20 all of the plants included in the fourth series of cuttings were taken from the pots; the soil was washed from the roots, after which they were examined thoroughly. Four out of the 16 plants had galls on the roots, only 1 of which was of any appreciable size.

(6) Cuttings were again made, this being the fifth set from the original galled plant. For checks, new daisy plants of the Queen Alexandra variety were purchased from a Boston firm and grown under the same conditions, so that both sets of plants would be about the same age when inoculated.

A fresh strain of the daisy organism was obtained in November, 1909, by plating from a gall, and inoculations were made December 1 on 31 of the supposedly resistant cuttings which were growing well and on 16 of the new daisies from Boston never before inoculated to be held as checks. The first subcultures from the poured plate colonies were used for the inoculations.

On December 14 (two weeks' time) galls had formed on 14 out of the 16 daisies of the new strain, but none whatever on the resistant strain.

On December 21 a gall had formed in one of the resistant cuttings; it was very tiny, but unmistakably a gall. By this time (end of third week) galls had formed on all the check plants and were from half an inch to an inch in diameter.

On January 6, 1910, 14 out of the 31 resistant cuttings had small galls starting to form. Some of these were merely a slight swelling. This was thirty-seven days after inoculating, and it will be remembered that all but 2 of the check plants had galls within two weeks.

On January 18 (forty-nine days) the supposed resistant cuttings were examined again and 23 of the 31 found with galls. None of the galls were larger than a small pea, however.

On February 9 all of the resistant cuttings had small galls, except 4, and 2 of these showed indications of swelling. This was seventy days after inoculating, and nothing comparable with this has been known to follow the inoculations of a daisy plant which had never before been inoculated with the gall organism. The beginnings of gall formation have been seen on daisy as early as the fifth day after inoculating, but the usual time for decided evidence is ten days or two weeks and always within three weeks.

On March 10, 1910, galls were forming on the 4 resistant cuttings which were still free from galls on February 9.

In July, 1910, all of the resistant plants bore large galls, i. e., growths 1½ to 2 inches in diameter.[a]

(7) Cuttings were made from these plants in August, 1910 (sixth set), and inoculations were made on these in November, December, and January, after they were well rooted and growing rapidly. The results are not yet ready to be reported upon.

So far as we have gone, loss of virulence may account for some of our failures to infect, but not, it would seem, for all, since in some of the experiments already described the check plants contracted the disease promptly, while the others did not. The results now under way ought to settle the question.

The following results are believed to be due, in part at least, to loss of virulence, but in part also to increased resistance. The weak point in the reinoculations is the almost complete failure of the checks.

In September, 1909, about 200 rapidly growing young daisy plants (rooted cuttings from old plants) were inoculated in the top of the shoot with young slant agar cultures of the old daisy gall organism (strain B).

No galls resulted. Thinking this complete failure might equally well be attributed to increased resistance on the part of the plants, since all of the cuttings had been taken from plants already twice and thrice successfully inoculated, the plants were repotted, top pruned, forced into rapid growth, and reinoculated.

The first reinoculations were on December 6, using young agar subcultures from several typical-looking colonies recently derived from a daisy gall by Miss Lucia McCulloch. The bacteria were pricked in. A small part only of the plants were inoculated. Checks were kept. All failed.

On December 13 to 17 the entire 200 plants were reinoculated by needle pricks, rather more than 400 groups of punctures being made on young branches. For this purpose young agar subcultures were used. They were derived from a colony recently isolated from a

[a] A comparison of No. 6 with earlier results seems to indicate that even when first isolated from a gall some colonies are more virulent than others.

daisy gall by Miss Brown and believed to be the right thing because it behaved typically on agar. The inoculations were made by the senior writer, assisted by Miss Bryan. Five days were devoted to the work, and, as 85 check plants were held, interesting results were anticipated, but no galls ever formed. The check plants (with two exceptions, 1020 and 1056),[a] also remained free, although they were in a growing condition and derived from plants never before inoculated and not long in the hothouse. The experiment must, therefore, be set down as a lost one without knowing quite why. Probably the failure must be ascribed to the use of a nonvirulent colony.

The plants stood in 10-inch pots, occupying the whole of a 125-foot, well-lighted greenhouse bench, and made throughout a good growth. They were of two susceptible varieties.

When the final examination was made in August, 1910, the plants were large and had been in bloom all summer. Occasional shoots showed a slight knobbiness where the needle pricks entered, and often there was more than the usual amount of corkiness in the pricked areas, but not a single tumor resulted from the inoculations. That these plants were still subject to infection (given a sufficiently virulent organism) is indicated by the fact that 13 of them bore natural tumors on the stem at the surface of the earth. Six of these tumors were large; the others were less than 1 inch in diameter. The parents of all of these plants (about 21 large daisies) all bore similar natural (and large) tumors on the base of the stem at the time the cuttings were made, and, as already stated, the plants from which they in turn were propagated had been (they or their progenitors) several times artificially inoculated with the production of galls. Cuttings were now made (August 5, 1910) from a large number of these plants for a second large experiment, and cultures were plated from the most favorable looking (youngest) of the 13 knots, with a view to obtaining a more virulent strain with which to make subsequent inoculations.

In November, December, and January inoculations were made on these plants as follows:

(1) With subcultures from a colony on a plate poured from the most favorable of the 13 tumors just mentioned.

(2) With subcultures from a colony on a plate poured from a daisy tumor occurring on a "nonresistant" plant.

Both these sets failed to produce tumors. Not only was this true of the "resistant" plants, but also of the check plants never before inoculated.

(3) Isolations were now made from a gall growing on one of Miss Brown's resistant plants (sixth series), and subcultures from two of

[a] These had very small galls in the inoculated places at the end of a year.

the colonies thus obtained proved to be actively virulent. When these were inoculated into the control daisies tumors soon appeared and are now growing rapidly. Numerous "resistant" plants were inoculated at the same time. All of these have developed small hyperplasias; but it is too early for comparative statements, and furthermore a correcter test, and one we have not yet been able to make (owing to the failure mentioned above), would be to inoculate checks and resistant plants with a virulent organism taken from a tumor on some plant *which had never before borne tumors.* This would remove the possibility of a heightened virulence in the organism used.

LOSSES DUE TO CROWN-GALL.

In consideration of the slow progress of this disease on many inoculated plants, the question has arisen whether crown-gall is really a serious disease or only to be regarded in the light of a minor disturbance, i. e., something comparable to warts or benign tumors in the higher animals.

Inasmuch as our exact experiments have not continued in all cases for a long enough period of years to give comprehensive results the most that can be done here in many instances is to summarize the opinions of growers and others who have given most attention to the disease as it prevails in the field, supporting these as best we may with our own observations, already detailed, in great part.

THE DAISY.

The plants are dwarfed and disfigured but only rarely killed outright or at least not for a long time. They are more or less stunted according to the size and rapidity of growth of the gall. Cuttings are injured worse than old plants. The New Jersey grower mentioned earlier is the only one who has made complaint to us.

THE ALMOND, THE PEACH, AND OTHER STONE FRUITS.

Toumey described this disease as serious on the almond in Arizona, and showed photographs of a 40-acre orchard ruined by it. Speaking of this orchard, he says:

> In the Glendale orchard some of the trees were diseased when planted. The actual number, however, that had galls upon them was very small. After the expiration of eight years, less than 1 per cent remained unaffected. * * *
>
> With each succeeding year a greater number of trees died outright or broke off at or just beneath the surface of the ground, where developing galls had gradually weakened the stem. A very conservative estimate would place the losses in this one orchard at at least ten thousand dollars. Probably the losses to the deciduous fruit and grape growers of Arizona from this disease amounts in the aggregate to from forty to seventy-five thousand dollars annually.

In reply to an inquiry, F. H. Simmons writes as follows (1910) concerning crown gall in Arizona:

There were 40 acres in the tract [probably Glendale orchard described by Professor Toumey]. I think they were set in the fall of 1889, and I took charge in 1899. The crown-gall was very bad on them, and in spring of 1897 there were cut and gathered three wagonloads of the gall. The trees were treated with bluestone on all cut surfaces. This treatment was followed up each year with less galls until spring of 1902 there was less than a bushel basket of galls cut. The drought by this time having made inroads on the trees the treatment was abandoned and part of the orchard pulled out, scarcely a gall being found. * * *

Trees badly affected seemed to have lost power of growth. There were practically on the mesa 125 acres in all. With the exception of 10 acres, all the orchards were badly affected, and about the year 1900 were practically out of business as a paying proposition, and have been nearly all pulled out.

Selby, of Ohio, reported to Toumey as follows:

From observations made in Ohio there seems no reason to believe that peach trees affected with crown gall at transplanting age will ever come to successful fruiting. * * *

One orchard in Lawrence County, containing 200 trees purchased in New Jersey, was grubbed out at seven years of age without having borne a single profitable crop, although other trees of like age situated near them had yielded fruit. These trees were badly affected when delivered, and were nearly all of them diseased at the time of removal. * * * Another parallel case occurred in Ottawa County.

In 1908 Selby made the following statement:

I do not recall a single instance out of many observed and recorded in which, the tree surviving transplanting, the removal of the galls by excision served to prevent the formation of new galls upon the same tree. Excision appeared to exert no influence whatever in the way of suppressing the trouble, and this irrespective of the location of the excised galls; whether but a single gall upon a small root or more than one gall on stem or root or both were removed and the wounds rubbed with sulphur, the new galls constantly appeared later. This may be taken as showing a diseased tendency of the plant tissues and this condition, this diathesis as it may be called, can scarcely contribute to the longevity of the tree independent of cutting off the water supply.

Earle reported to Toumey as follows:

Crown-gall is very abundant in Alabama on the peach and is sometimes found on the plum. I consider it a very serious peach disease in Mississippi and Georgia, as well as in this State.

In 1892 Wickson, of California, wrote as follows:

For some time many nurserymen followed the practice of removing the knots from the trees as dug from the row, but this was abandoned when it was found that the knot commonly reappeared after planting in the orchard. At present no reputable nurseryman sells such trees; they are burned at the nursery.

Probably during the last twenty years hundreds of thousands of such trees have spindled and died in the best soil and with the best treatment.

Woodworth, of California, reported to Toumey as follows:

The crown gall occurs in California on all our deciduous fruit trees and on grapes. It has been abundant and serious.

Toumey wrote:

In California, where the fruit industry is many times what it is in Arizona, the losses must be correspondingly great.

In Pennsylvania on fruit trees in the nursery, according to Butz (Ann. Rep. Pa. State College, 1902, p. 405):

There is little warning of the presence of the disease in a block of trees while they are developing into salable stock, but when they are taken up it is frequently discovered that from 20 per cent to 80 per cent of them are affected at the roots with crown-gall, rendering them unsalable.

Butz also cites from correspondents as follows:

We have known peach blocks in New Jersey to be entirely destroyed. * * * One year ago we had it bad in peach and threw away thousands.

APPLE TREES.

Whitten, of Missouri, reported to Toumey as follows:

I have seen it on a few apple trees in the nursery, but it was not severe enough to impair their growth.

Concerning the injury done to orchard trees, Butz has the following as the result of one of his experiments.

On November 21, 1898, 11 apple trees were planted upon the station grounds. These trees were donated by a Pennsylvania nurseryman, and all of them bore galls at the crown varying in size from a hickory nut to an unhulled walnut. The root system of these trees was apparently most excellent, having an unusual amount of fibrous roots. But owing to the fact that these fibrous roots proceeded mainly from and about the galls it was evident that the galls were the inciting cause of the unnatural development. The trees were three years from the graft, and but for the galls were excellent trees for planting in the orchard. Five of these trees were York Imperial and six were Ben Davis, the two varieties of apple which are most susceptible to crown-gall and the most extensively propagated and planted in Pennsylvania. Records taken in April, 1901, after the trees had made two seasons' growth, show immediate injury due to the galls. Two trees of York Imperial had died, and the other three had made only weak and slender growth. * * *

Of the Ben Davis trees, all grew, though the growth made was in all cases short and weak. The length of the best shoots made in the second season varied from 4 to 10 inches. After another year's growth these trees are still living, making some new wood each year, though it is not as strong as it should be. An examination of the galls at the roots (June, 1902) by removing the ground about them shows that they are increasing in size, and in some cases more completely girdling the trees than when they were planted. The effect of this gall development is shown in the heavy production of sprouts from the stock roots below the gall and the consequent weakness in the graft head. * * *

A peach grower in Franklin County in Pennsylvania is now having a similar experience with peaches. He wrote me in November, 1900, that he suspected something wrong with a block of 1,000 peach trees in an 80-acre orchard, and digging at the roots discovered an enlargement which was identified at this station as crown gall. The trees came from an Alabama nursery and were planted in the spring of 1899. The growth during the first two years was excellent, but now as the trees reach fruiting age they indicate a weakness that can not be overcome.

He also cites from a correspondent as follows:

It is more prevalent in apple than in anything else. On the block of apple trees which were 2 years old when you were here, we did not find a single tree affected, while on our trees, now 2 years old, we find 30 to 40 per cent affected with crown-gall and we will sustain a big loss. At the time these 2-year trees were grafted, I grafted 30,000 for a neighbor for his own orchard planting and on the trees taken up he has found but 2 or 3 per cent affected, though the source of stocks and grafts was the same. This looks as if the disease was in my ground.

The conclusion of this nurseryman is entirely correct; the cause of the disease is in his ground.

A former colleague, Mr. P. J. O'Gara, who has had a very wide experience on the Pacific coast, has observed the disease to be seriously injurious to Spitzenberg apples in Oregon, and also to pears, dwarfing the trees and reducing the size of the fruit. He states that hold-over blight (*Bacillus amylovorus*) is very apt to find lodgment in the galls when they occur above ground and that root-rot begins commonly in the galls when they are underground (oral communication). He is also our authority for the statement that crown-gall has seriously injured peach growing in Colorado. The disease seems to be worse in dry climates, where irrigation is practiced.

In 1910, after conversation with Mr. O'Gara, the following letter was received from him:

I am inclosing a photograph of crown-gall (hairy-root type), taken in my office at Medford, Oreg. This tree is 7 years old, but is no larger than a good 3-year old and certainly not so vigorous. This tree is exactly like 50 trees in the same apple orchard, the variety being Esopus Spitzenberg. Crown-gall, either *hairy*, *hard*, or *soft* types, certainly injures apples if the infection starts with the seedling or the graft. If a tree is several years old before becoming infected, serious injury is not so liable to be the case, as the vigor of the tree somewhat counteracts the effects of the gall. But Spitzenberg apples infected on bodies or crowns often become so "warty" that growers cut them out. Besides, crown-gall above the ground always permits the entrance of fungi, and in susceptible varieties like Spitzenberg, *Bacillus amylovorus* gets in its deadly work through the gall. Anyone having experience on the Pacific coast knows that a crown-gall above the crown of a Spitzenberg means blight infection sooner or later.

Later Mr. O'Gara sent on a blighted apple limb from Medford, Oreg. (Pl. XXXI), with the following note:

I am sending you under separate cover a specimen of Spitzenberg apple limb which has a bad crown-gall, through which pear blight infection entered. Crown-gall on the body or crown of a Spitzenberg apple is very dangerous, from the blight standpoint. The past year I have seen hundreds of blight infections through these galls. For this reason every crown-gall must be removed, and our inspectors enforce this regulation to the letter.

In 1898 Selby cited the case of a grower of nursery stock who found part of a block of apple trees badly affected with gall about the year 1893. The trees were dug up and the ground left to rest a year,

then peach trees were planted. In that portion where the apple trees had been diseased most of the peach trees became affected with galls, and were worthless.

Quite opposite views are expressed in the following citations, the first one of which is from Mr. F. C. Stewart, of the experiment station at Geneva, N. Y. (Proc. 53d Ann. Meeting, West. N. Y. Hort. Soc., Rochester, Jan. 22 and 23, 1908, p. 98):

In this connection it should be mentioned that the crown-gall of apple, although resembling crown-gall of peach and raspberry, is an entirely different thing.[a] There is abundant proof that the apple crown-gall is not communicable from one tree to another. Moreover, in New York, at least, apple crown-gall is an unimportant disease. Although common in our nurseries, it is rarely found in orchards. In 1899 C. H. Stuart & Co.,[b] Newark, N. Y., set out an experimental orchard of 500 trees, mostly Baldwins, all affected with crown-gall. The trees have now been set nine years. Under date of January 20, 1908, Mr. Stuart writes as follows: "These trees to-day show as good a growth as the trees planted the same time and free from crown-gall. The bark is smooth, healthy in appearance, and the trees look thrifty and vigorous." An experiment made by the station bears on this point. In 1901 we planted 22 apple trees affected with crown-gall to determine the effect of this disease upon the growth of the trees. The trees were 3 years old. The galls varied in size from 1 to 2 inches in diameter and were located mostly on the taproot, but in a few cases on lateral roots. Some of the trees had several galls each. We believe the galls were typical of those commonly found on apple trees in New York nurseries. Five of the trees were dug in 1903, 5 in 1905; and the remainder in 1907. In no instance was there any evidence that the galls had increased in size or number, or that they had been in any way injurious to the trees.[c] Probably apple trees bearing large galls should be rejected, but unaffected trees from the same lot may be planted without fear of bad results.

Mr. Barden also writes as follows to Mr. George G. Atwood, chief bureau of horticulture, Albany, N. Y., concerning this same orchard:

Referring to yours about crown-gall on nursery trees that have been planted in orchard for several years, I would say that the Stuart orchard on the Bailey farm 3 miles north of Newark is the only one that I have had any knowledge of. In company with Mr. Stuart I drove to this farm last fall [1909] and carefully studied the different trees, every one of the 400 [d] having been planted with a large crown-gall on it. These trees have now been planted eight years, and, with the exception of a few that were girdled by mice several years ago, are in a vigorous and healthy condition.

The growth has been even, no stunted trees, and it would certainly be hard for an orchardist to condemn a tree on account of crown-gall after seeing this orchard.

Doctor Hedgcock also regards crown-gall as of small consequence to the apple, especially if the root-grafts are well made. His field experiments on the apple have been extensive (mostly in the Mississippi Valley), and cover a period of five years. Mr. Güssow has expressed similar views.

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[a] See note under raspberry.

[b] Nurserymen.

[c] The location of a gall perhaps may determine its injuriousness, i. e., whether on crown or root. Butz's trees bore galls on the crown. So far as known, no comparative orchard tests have been made.

[d] Five hundred in Mr. Stewart's statement. Were 100 lost during these years? And if so, how many by crown-gall? No checks appear to have been held for comparison.

THE QUINCE.

The galls of the quince (*Cydonia vulgaris*) occur on the stems, and are warty in appearance. Often an entire limb will be covered by these broad irregular outgrowths. Whole orchards in California have been attacked by these galls and quince trees in other western States are known to be affected. Mr. Hedgcock has received diseased specimens also from Ansted, W. Va. Doctor Trabut sent specimens of quince gall from North Africa (Pl. XXXV). Lounsbury reports a quince gall which appears in the form of "rough, lumpy growths" as common in South Africa.

THE RASPBERRY AND THE BLACKBERRY.

The disease appears to be quite prevalent on the red raspberry in various places in the United States, and must be regarded as injurious, although some nurserymen are of a contrary opinion. The extent of injury to black raspberry and to the blackberry is not known. Mr. P. J. O'Gara has observed one apple and pear nursery in Oregon where practically all of the young trees were galled. This nursery was set on the site of an old berry patch in which the crown-gall had prevailed (verbal communication).

The following similar statement is taken from the report of the Dominion Botanist (Güssow) (1 George V, Sessional Paper No. 16, A. 1911, p. 273):

> One prominent grower had a small area planted with raspberries. These on being taken up showed many "root galls." The plants were destroyed and no specimens were sent us for examination. The grower then planted a large area to young peach trees, the rows of which passed through the land formerly occupied by the raspberries on which the root galls were discovered. He then observed that the peaches growing on this latter area were not doing well and finally failed, while all the other trees did exceedingly well. On taking up the failing peach trees, their roots showed plenty of root galls, while the others growing outside the raspberry area were free from it. The same facts were recorded by other growers. There could hardly be given a more typical example of an infectious disease. But, unfortunately, we were not acquainted with any of these observations until it was too late to make any investigation. If these facts as related are correct, and we have no reason to doubt them, there is still a considerable amount of research necessary.

Selby is on record as long ago as 1898 to the same effect. He says that 16 per cent of some healthy peach trees planted in a badly galled raspberry plantation became affected with the gall.

Wulff's statements (Studien über heteroplastische Gewebewucherungen am Himbeer- und am Stachelbeerstrauch, Arkiv für Botanik, Bd. 7, No. 14, Upsala, 1908) are equally explicit. He says respecting the appearance of the raspberry gall in a garden near Karlshamn (South Sweden):

> On an area of 33 by 4 paces were about 100 raspberry bushes, all very badly affected by the disease. * * * From the time of their planting in 1901 to the summer of

1907, inclusive, the bushes were always sick, and have during the whole time borne either no fruit whatever or a very scanty crop.

These plants were an ever-bearing variety from Denmark.

In August, 1907, Wulff also found a bad outbreak of the disease in middle Sweden near Orebro:

Here about 800 bushes of Red Hornet and about 100 of Superlative were attacked. The first-named bushes were planted in 1901, had borne very well during the first years, and appeared entirely normal. In 1906 the first symptoms of the disease were discovered, and in consequence of this no crop was borne in the summers of 1906 and 1907.

In the next paragraph Wulff speaks of the disease as "very injurious to raspberry culture" everywhere in Sweden where it has appeared. He also brings forward evidence to show that frost injuries have nothing to do with its appearance, and cites similar statements by Blankenhorn and Mühlhäuser (vide Sorauer I, 596) with respect to the grape gall. Wulff's own statement is:

Bei meinem Untersuchungen der Himbeerkallose habe ich niemals auch nur die geringsten Andeutungen von Frostbeschädigungen entdecken können.

Concerning the origin of the disease neither in this paper nor a second one (Weitere Studien über die Kallaskrankheit des Himbeerstrauches, Arkiv für Botanik, Bd. 8, No. 15, Upsala, 1909) does he reach any positive conclusion, other than that he has not been able to find in the fresh overgrowths any parasitic organism and is inclined to ascribe them to excessive nitrogen nutrition and excessive water supply.

Lawrence (Some Important Plant Diseases of Washington, Bull. No. 83, 1907) shows a very interesting figure of blackberry canes split open by the growths arising from within and says that in the State of Washington the disease is very destructive to the Snyder, and that occasionally Kittatinny and Himalaya Giant are badly infected, while Erie, Early Harvest, and Evergreen are not seriously injured.

He has also observed the disease to be severe on the red raspberry, especially the form growing on roots and crowns.

Güssow has attributed a gall on the blackberry in England to a fungus, *Coniothyrium tumefaciens* n. sp.

THE ROSE.

Occasionally the disease is very prevalent on the roots of roses grown in the hothouse, and skilled gardeners are generally of the opinion that the galls are seriously injurious, reducing the size of the plants, the amount of foliage, and the vigor of the flowers. Here again exact comparative studies are wanting. It must be obvious, however, in the case of a small plant like the hothouse rose, that the

energy used up in the production of the galls, which are often large, must be abstracted from the general needs of the plant, which as a result must either yield an inferior product or blossom for a shorter period.

The following statements were received in 1909–10 from a rose grower who had much of the gall in his houses:

Our houses of 10,000 plants seem all to be affected, and it looks [October 23] as though we would have to throw the plants out.

The disease was definitely identified as crown-gall by the writers, who received numerous well-developed specimens (Pl. XX, fig. 2) and recommended substitute crops. Nematodes were not observed. This man was asked later in the season for more definite information concerning his losses and replied as follows:

Replying [February 22] to yours of 16th instant, would say that after consultation with other growers of roses who had had experience with crown-gall and eelworm, we decided to keep our plants in and get what we could from them, rather than take a chance on some other crop so late in the reason.

All the plants are affected more or less—some not as bad as others—while perhaps 200 or 300 have been killed outright.

The great loss is shown when we come to cut the buds. At a time when we should have been cutting 1,500 to 2,200 a day, we were cutting but from 400 to 600, and the average loss for the season thus far has been on a conservative estimate 67 per cent.

We will cook our soil this year and hope for better results another season.

In December, 1910, this grower wrote as follows:

Replying to yours of 9th instant, would say we did cook our soil last spring, as we wrote you we should, and that we have had *no trouble* with crown-gall this season.

Our plants are very fine this year, and we have been cutting some very fine blooms. Just now we are off crop, but plants are breaking in good shape and the future looks very promising.

Our commission house sent us word early in the winter that they had not seen finer specimens of Bride outside of the flower show than the ones we were shipping.

THE GRAPE.

European observers have generally regarded the scab of the grape as a serious disease.

Delacroix (1908) states that the attacked shoots grow feebly for a year or two and then the parts above the galls dry out and die.

The statement of Cavara respecting rachitic growth has already been quoted (p. 15).

In Italy, in 1906, in the Po Valley (near Modena), the senior writer saw cases of *rogna* on large vines and was informed by competent viticulturists that the disease was becoming more and more prevalent, mostly on the flat irrigated lands, but to some extent also in the hills, and that the life of an attacked vine seldom extended beyond four years. In sections of Italian rogna of the grape preserved in 10 per

cent formalin the senior writer saw bacteria in the browned outer crevices much like those described by Cuboni (1.5×0.3 to $0.5\ \mu$), but less numerous and not likely to be the parasite.

RED CLOVER.

Galls have been found on roots of red clover (*Trifolium pratense*) in Kentucky and Alabama. It is not yet known how destructive this organism is when it gains entrance to a clover field.

ALFALFA.

Roots with tubercles other than the nitrogen-fixing nodules have been found on alfalfa plants (*Medicago sativa*) in Kentucky, Maryland, Pennsylvania, Alabama, and New York (?). The galls are found on plants in fields where the stand is very poor and also an occasional gall is found on plants in very good fields. The plants affected do not grow to full size, but it is not yet known whether they are killed directly by the work of the gall organism or not, although large portions of fields die and the roots are found more or less affected with galls.

COTTON.

The crown-gall of the cotton plant (*Gossypium* sp.) occurs rarely (so far as our information goes) and is not known to cause any trouble whatever to the growers of cotton. It has been found in Texas and also on the crown of cotton plants growing in the greenhouse in Washington.

HOPS.

The reports of hop growers on the Pacific coast indicate that this disease may do considerable damage, particularly as the galls often reach a diameter of one's double fist. Some believe that an attack of two years' duration is sufficient to kill a plant. According to Dr. W. W. Stockberger, of this Bureau, the disease occurs on hops not only in Washington State and Oregon, but also in the Sacramento valley in California: "There I have seen acres of hops in which scarcely a hill could be found which did not show these tumors, some of them being larger than my fist."

SUGAR BEETS.

A crown-gall also occurs naturally on the sugar beet both in this country and in Europe. While rather rare in the United States, it appears to be widely distributed, and more common some seasons than others. We have received specimens from localities as widely separated as Virginia, Michigan, South Dakota, Utah, California,

and Washington State. In general it is easily distinguished from the attacks of nematodes (Pl. IV, fig. 1). It is less easily distinguished from what we have called tuberculosis of the beet. The latter occurs in Kansas and Colorado. It appears to be most prevalent in Colorado where at least one field was badly injured. According to one of our correspondents it is on the increase. Should this disease become widespread the yield of sugar would be greatly reduced.

Crown-gall seems to be rather infrequent in Germany, judging from Dr. Reinelt's paper in Blätter für Zuckerrübenbau (Berlin, 31 März, 1909), since with the assistance of various sugar-beet men he obtained only 47 specimens for his studies.

According to Dr. Kølpn Ravn, of Copenhagen (oral communication), the gall occurs on sugar beets in Denmark, but does not injure the crop, only about one beet in a million showing it.

Of 3,247 beets dug in November, 1910, in Virginia (Arlington Experimental Farm), 5 bore tumors.

The galls on the beet often grow to large size, e. g., Reinelt mentions some as large as a child's head or larger (weight 1.5 kilos), others which caused thickenings of the whole or a great part of the root, and still others which were small as peas, but set close together over the whole surface of the root.

This gall we believe to be due to the crown-gall organism. Three times prior to 1910 typical looking colonies on agar poured plates were obtained from the interior of beet galls from California and once from Virginia. The Virginia colonies were not transferred to subcultures, and the two or three colonies selected from the California plates proved nonpathogenic to sugar beets; no additional opportunity for making poured plates occurred until November, 1910. (See pp. 81-85.)

Reinelt failed to isolate bacteria from the inner tissues and comes to the conclusion that bacteria are not present. He used various sorts of gelatin media. His technique of surface sterilization appears to have been proper and the source of his failure appears to have been (1) that he selected improper material (too old), (2) that he did not wait long enough for the bacteria to appear on his plates, or (3) that he diluted his infectious material too much. The period the plates were under observation is not stated. He should have held his plates for at least ten or fifteen days; he should also have mashed up the fragment of beet and inoculated copiously from the first tube, whereas he did not crush his material but only allowed the small cube to remain in the bouillon for a short time and then made his inoculations from a third transfer (third tube). Judging from our own experiments, on daisy galls, the third tube of bouillon prepared in the manner he describes would ordinarily contain very few living

bacteria—often none, or less than 1 per loop (see p. 168).[a] If he had mashed his cube in the first tube of bouillon, allowed the contents of the crushed cells to diffuse for an hour, and then inoculated directly from this first tube, *rather copiously*, e. g., with several 3 mm. loops of the fluid, he would probably have had colonies of the

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[a] As the result of poured plates made in 1910 by Lucia McCulloch, using a sound old hop gall received from the Pacific coast, it would seem that there were less than 500 living bacteria per cubic inch of the material used. The right organism was plated out and tumors obtained with it on sugar beet and daisy, but two of the three colonies selected were noninfectious.

Plates of +15 nutrient agar, poured by Miss Brown in the fall of 1910 from tumors on sugar beet, gave the following results:

(1) First set of Arlington (Va.) plates. Two c. c. of a rather old and tough tumor were mashed in 10 c. c. of bouillon. Eleven plates were poured, all from the original tube, inoculating as follows:

3 plates each five 3 mm. loops.
3 plates each four 3 mm. loops.
2 plates each three 3 mm. loops.
2 plates each two 3 mm. loops.
1 plate one 3 mm. loop.

Five favorable colonies appeared on this set of plates.

(2) Second set of Arlington (Va.) plates made from another tumor—material good. Three c. c. were mashed in 10 c. c. of bouillon. Eight plates were poured. The first six were from the original tube, the other two from the first dilution. The inoculation was heavy, viz:

2 plates with five 3 mm. loops.
2 plates with four 3 mm. loops.
2 plates with three 3 mm. loops.
1 plate with three 3 mm. loops.
1 plate with two 3 mm. loops.

Fifteen favorable colonies appeared on this set of plates.

(3) First set of Blissfield (Mich.) plates.

Of this tumor 3.4 c. c. were mashed in 10 c. c. of bouillon. Eight tubes were poured, the first six from the original tube, the other two from the first dilution, inoculating as follows:

3 plates each with three 3 mm. loops.
2 plates each with two 3 mm. loops.
1 plate with one 3 mm. loops.
1 plate with two 3 mm. loops.
1 plate with one 3 mm. loop.

Five colonies resembling gall colonies came up on this set of plates.

(4) Second set of Blissfield plates (same tumor, next day), using 0.5 c. c., which was mashed in 10 c. c. bouillon. Eight plates were poured, all from the original tube, inoculating as follows:

4 plates each with four 3 mm. loops.
1 plate with three 3 mm. loops.
1 plate with two 3 mm. loops.
2 plates each with one 3 mm. loop.

No gall colonies appeared on this set of plates.

(5) First set of Fairfield (Wash.) plates. A smooth tumor 3.5 to 4 cm. in diameter was selected and about one-half of it (possibly 10 c. c.) was mashed in 10 c. c. of bouillon for the plates. All of the eight plates were poured from the original tube, inoculating as follows:

5 plates each with five 3 mm. loops.
2 plates each with four 3 mm. loops.
1 plate with two 3 mm. loops.

No gall colonies appeared.

(6) Second set of Fairfield plates (same tumor). About one cubic centimeter was mashed in 10 c. c. of bouillon. Eight plates were poured, all being inoculated copiously from the original tube, viz:

4 plates each with five 3 mm. loops.
2 plates each with three 3 mm. loops.
2 plates each with two 3 mm. loops.

Four colonies looking very much like the gall-forming organism grew on these plates.

(7) Plates were also poured in December from a gall on another Arlington beet which had been transplanted to the hothouse for six weeks. These yielded only one colony resembling *Bacterium tumefaciens*, and this gave no positive result when inoculated into sugar beets.

Of these 30 colonies, as already stated, only 5 have proved infectious and all of them are possessed only of feeble virulence.

(For a quantitative study made by the senior writer in November, 1910, see under *"Sugar beet,"* p. 81.)

gall organism in all of his plates, provided nothing was wrong with his culture medium or the galls themselves were not too old. We have not used gelatin media for isolations from galls, but ordinary +15 peptonized beef-bouillon agar.

Dr. K. Spisar has also investigated the sugar-beet gall and reaches the conclusion that it is not due to animal or plant parasites of any sort (Zeits. f. Zuckerind. in Böhmen, Prag., Aug., 1910). Bacteria do not occur in all the galls and with those he cultivated out he could not reproduce the disease. He, therefore, ascribes it to wounds, but does not advance any satisfactory reason why it should arise in some wounds and not in others.

Since the above paragraphs were written we have plated what we believe to be the right organism from natural tumors on the sugar beet and with subcultures therefrom have obtained small slow-growing galls on beet (Pl. XXXVI, fig. 1), tomato, and daisy. Most of the colonies tested were noninfectious.

TUBERCULOSIS OF BEETS.

In the autumn of 1910 beets from Colorado and Kansas were found commonly attacked by a yellow schizomycete capable of causing cells to proliferate in a nodular growth. On section the attacked parts showed as small, water-soaked, brownish areas (Pl. XXXIV, fig. 2). Under the microscope great numbers of bacteria were observed therein and the center of the spot was seen to be disorganized into a small cavity. Often the surface of the nodules bore small central radiating fissures (Pl. XXXIV, fig. 3). The appearance of these cracks suggested the possibility that they preceded the infection. In some instances these brownish areas of softening were traced from the galled portion of the beet into the ungalled part. The diseased parts appeared mucilaginous—stringy when touched.

This disease, which was at first supposed to be crown gall, is only superficially like the latter, because, as in the olive tubercle, the bacteria are abundant and easily detected and produce areas of softening and central cavities. The disease has been reproduced on sound sugar beets in the department hothouses by pure-culture inoculations (subcultures from poured plate colonies).

From these artificially produced tubercles the organism has been reisolated and successfully reinoculated into other sound beets. Up to this time cross-inoculations on other plants (daisy, tomato, etc.) have failed.

Description of Bacterium beticolum n. sp.—This organism, which may be known as *Bacterium beticolum* n. sp., is a rod with rounded ends, single or in pairs, chains or clumps. Clumps and chains frequently occur, especially in pellicles. It measures about 0.6 to 0.8

by 1.5 to 2.0μ. It is flagellate by means of several polar flagella. No spores have been observed. It has a capsule. It liquefies gelatin slowly, but not Loeffler's blood serum. Gelatin stabs at 18° C. required a month for complete liquefaction. It reduces nitrates. It grows readily in peptonized beef-bouillon containing 9 per cent sodium chloride. In ordinary peptone bouillon there is uniform clouding and a copious pellicle, which falls easily. It is killed in beef-bouillon by 10 minutes' exposure in the water bath to 51° C. It grows at 37° C., but not so well as at room-temperature (bouillon). It also grows slowly at 1° C. in bouillon. In milk the growth occurs mostly on the surface. It forms a yellow rim and pellicle and slowly solidifies it, but the whey separates very slowly. The fluid is viscid. Litmus milk is blued, and subsequently reduced (1 month). After boiling, the color returns red. It does not grow in Cohn's solution. It grows readily in Uschinsky's solution, making it viscid, like *Bacterium pruni*. In this fluid rods with enormously thick-walled capsules occur. It makes a moderate growth on potato. It does not convert the fluid around the cylinder into a solid slime. There is a copious starch reaction with iodine even after many weeks' growth.

For experiments in fermentation tubes a basic solution was made of river water containing Witte's peptone. In this the following carbon compounds were tested: dextrose, saccharose, lactose, maltose, mannit, and glycerin. The organism grew readily in the open end of all the tubes and clouded the closed end except when lactose and glycerin were offered to it. No gas was produced from these carbon compounds. It did not produce gas in any culture medium, except possibly sparingly in beef peptone gelatin. It should be tried for gas formation in presence of inosit. On thin sown agar plates the colonies may become 1 cm. in diameter. Often they are smaller. These colonies are circular, smooth or wrinkled. The colonies are similar on gelatin and finally form saucer-shaped liquefactions, or if the plate is thickly sown the whole becomes fluid. It grows well the whole length of agar stabs, and sometimes sends out small brush-like projections. Growth is much paler in cane sugar agar, but becomes yellow with age. Indol is produced in 2 per cent peptone water, but less abundantly than by *Bacillus coli*. It grows readily in bouillon over chloroform. It is not killed by drying (fourteen days). It stains well by Gram. It is yellow or becomes yellow on all ordinary culture media.

SHRUBS, SHADE TREES, AND FOREST TREES.

We have no means of determining the amount of injury done by crown gall to nut trees, shade trees, etc. The disease is common on the chestnut and the gray poplar in the eastern United States, and is said to occur frequently on the Persian walnut in California.

Under date of September 24, 1909, Mr. Frank N. Meyer, agricultural explorer for this Department, sent from Angers, France, a young plant of *Arbutus unedo* bearing root galls. From these galls bacterial colonies resembling the daisy organism were plated out and galls produced on sugar beet by pure-culture inoculations (Pl. XXIV, *A*).

Lounsbury has reported it, or something closely resembling it, as prevalent and injurious on the willow in the Transvaal and Cape Colony, South Africa, where it appears to be a new trouble, having come to scientific attention first in 1899. He sent some of these willow galls to us and from one of them a colony was plated which produced slow-growing galls on the daisy and upon weeping willows (Pl. XXXV, fig. 1).

HOTHOUSE PLANTS.

Other than those already mentioned we have found what may be this disease on roots of lettuce (Pl. XXXVI, fig. 2). Our attention was called to this by Mr. W. W. Gilbert, a Bureau colleague, who turned the material over to us with the statement that he could not find any nematodes in the root swellings. We also failed to find them. Thereupon poured plates were made.

The plants shown on the plate were photographed natural size. They had been growing nearly three months and were badly dwarfed. There were many such plants in the hothouse and all had similar galls on their roots, and no other assignable cause for their stunted appearance, since the roots of those lettuce plants in the same house which had made a good growth were free from these nodules. The only other disease in the house was an occasional case of the *drop*.

Agar-poured plates were made from one of these galls after properly sterilizing the surface and colonies obtained which resembled those of *Bacterium tumefaciens*. With subcultures from half a dozen of these colonies inoculations have been made into the roots of young sugar beets, but no galls have appeared to date (13 days).

BEST METHOD OF DEALING WITH THE DISEASE.

Up to this time the best method of dealing with this disease remains the old one of strict inspection of nursery stock and the condemnation of all trees and shrubs found diseased. In individual cases this undoubtedly works hardship to the nurseryman, but, on the other hand, to allow him to sell galled trees injures the fruit grower, serves to distribute the infection broadcast, and tends to destroy his own reputation. The nurseryman's remedy lies in careful methods and the abandonment of infected soils.

By no amount of special pleading can it be made to appear that an infectious disease should be tolerated on nursery trees offered for sale simply because it is rather prevalent and is inconvenient to deal with. Before the nurseryman can be allowed to sell such trees without restriction he must establish conclusively that it is not injurious, and not transmissible to susceptible species.

We are disposed to include apple trees also in this recommendation. While these seem to be less subject to crown-gall in a serious form than some other plants, frequently they do not make good trees, and our cross-inoculations suggest, at least, that they may serve to carry the disease to other plants and into localities previously free from it. Moreover, even when the apple gall does not itself seriously injure the tree it may serve, as we have seen, for the entrance of other parasites.

In some cases the inspector will be in doubt whether to condemn stock or pass it, particularly when the trees have been carelessly grafted and show more than the ordinary amount of callus. He may then either refer the specimens to some more experienced pathologist or refuse to take chances. Until we know to the contrary excessive callus should be regarded as incipient gall. Ordinarily there will be no difficulty in determining whether or not a given lot of trees has crown-gall or hairy-root, except when the nursery stock has been dishonestly pruned before shipment to remove signs of the disease, and then usually some traces will be left. In case trees are improperly condemned there is always a remedy at law.

SYNOPSIS OF CONCLUSIONS RESPECTING CROWN-GALL.

(1) Crown-gall is a disease common in nurseries on the roots and shoots of various plants, and likely to continue on the plants when they are removed to orchards, vineyards, gardens, and hothouses. It also occurs on various field crops. This name is used for the disease whatever the situation of the galls on the plant.

(2) When we began our studies the cause of crown-gall was unknown, and by them it has been determined.

(3) Bacteria were seen in crown-galls of the daisy in 1904, and the studies then undertaken have been pursued continuously to date, and are here first offered in complete form.

(4) The first successful isolations and infections were obtained in 1906, and the biology of the bacterial organism derived from the daisy has been determined more carefully than that from galls on other hosts.

(5) Hundreds of pure-culture inoculations on daisy have removed the subject from the domain of speculation and shown that the galls

on Paris daisy are due to a white schizomycete named *Bacterium tumefaciens* (April, 1907).

(6) This organism is a short rod multiplying by fission and motile by means of polar flagella. It can be grown in many sorts of culture media, but does not live very long upon agar. It forms small, round, white colonies in agar or gelatin poured plates. Under unfavorable conditions of growth it readily develops involution forms.

(7) This schizomycete differs from many bacterial organisms in not producing open cavities in the plant. It appears to occupy the living cells in small quantities, causing rapid proliferation.

(8) We have not been able to stain it in the tissues, at least not satisfactorily.

(9) It is readily plated from young sound galls, i. e., those not fissured or decayed, often in practically pure culture, but it comes up slowly on +15 nutrient agar, and generally not very abundantly. It grows, however, promptly on agar when transferred from cultures.

(10) It produces galls most readily in soft, rapidly growing tissues. Ordinarily, resting tissues can not be made to produce galls. Turnips seem to be an exception.

(11) Cross-inoculations to plants of other families have shown the daisy organism to be capable of inducing tumors on many species in widely separate parts of the natural system (Compositae to Salicaceae), these galls varying somewhat in appearance.

(12) Some species of plants were not infected (onion, fig, olive) and possibly are not infectable, but further experiments should be made.

(13) For purposes of comparison natural galls have also been studied on the following plants: Peach, apple, rose, quince, honeysuckle, *Arbutus unedo*, cotton, poplar, chestnut, alfalfa, grape, hop, beet, salsify, turnip, parsnip, lettuce, and willow.

(14) From all of the preceding, by means of Petri-dish poured plates on agar, schizomycetes have been isolated closely resembling (as grown on agar) the *Bacterium tumefaciens* obtained from the Paris daisy.

(15) With eight of these organisms tumors have been produced on sound specimens of the species from which obtained. With these eight and two others (not tested on the host) tumors have been produced on daisy and various other plants, thus tending to show a wide range of natural cross-inoculability.

(16) On pages 133 and 137 the reader will find tables summarizing all the results of the inoculations.

(17) These organisms have been studied comparatively as to their morphology and cultural characters and found to differ only slightly from each other, and from the organism isolated from the daisy, i. e., the agreements are more conspicuous than the differences.

(18) The beginnings of the galls are visible in some cases as early as the fourth day after inoculation by needle prick, and they often reach a large size in one to two months, but frequently on woody plants they continue to grow for several years. On the contrary, sometimes they have been very slow to develop.

(19) Some cross-inoculate less readily than others, but in general the monotonous morphology, the cultural uniformities, and the ready cross-inoculability (daisy, peach, hop, grape, poplar, alfalfa), point to one polymorphic species rather than to several distinct species, but further studies should be made.

(20) The galls are often rapidly invaded by saprophytic bacteria, especially the softer galls. On agar poured plates many of these bacteria are readily distinguished from the parasite by differences in form and color, but others are distinguished therefrom with great difficulty, cultures on other media or inoculations being requisite.

(21) The galls also invite various parasites—nematodes, fungous root rot, fire blight of apple and pear, etc., and some of these are able to cause great damage.

(22) We have not been able to distinguish etiologically between *hard* galls and *soft* galls. Even the hardest crown-galls are due to bacteria which closely resemble those found in the softest.

(23) Overfed plants are more subject to the disease than those making a moderate growth.

(24) The size of the tumor, other things being equal, depends on how rapidly the plants are growing, i. e., the state of nutrition. Actively growing plants usually developed large tumors when inoculated, and slow-growing plants none at all or small ones; but, as in apple, small slow-growing galls may finally become large. This long-continued growth would not be possible if there were not a very nearly even balance between the stimulus of the parasite and the response of the host.

(25) The apple hairy-root, hitherto a disease of unknown origin and supposed to be noninfectious, has been shown to be due to bacteria which culturally and morphologically differ, if at all, only slightly from the crown-gall organisms.

(26) This causal organism is located not in the hairy roots themselves but in the flattened tumor from which such roots arise.

(27) Typical hairy-root has been produced on sound apple seedlings by pure-culture inoculations, and in the same way on sugar beet both galls and hairy-roots have been obtained.

(28) These abnormal growths which we have designated indifferently as tumors or galls are believed to be like malignant animal tumors in various particulars: Permanent and very rapid new growth containing all the tissues of the part attacked; enormous round-

celled or spindle-celled hyperplasia; great reduction of amount of conductive tissues; early necrosis, especially of the more fleshy tumors, with renewed growth at the margins; frequent recurrence after extirpation; extension of the disease to other parts by metastases, etc.

(29) The disease is one which progresses slowly, stunting the plant first and finally destroying it, unless removed by extirpation or by the development of increased resistance on the part of the plant.

(30) The continuation of rigid State inspection with rejection of diseased nursery stock is recommended.

(31) The organism is moderately susceptible to germicides but can not be reached in the galls. Moreover, germicidal treatment, after excision of the galls (p. 184), can not be depended upon in all cases because of the tendency of the organism to form metastases.

(32) The organism from the daisy loses virulence on culture media, and in some cases is believed to lose it also in the tumor itself (daisy, hop, sugar beet).

(33) The organism is believed to occur inside the rapidly proliferating cells, which by its presence are stimulated to divide with formation of the tumor.

(34) During the progress of our studies a new disease of the sugar beet has been discovered. This disease, which is liable to be confused with crown-gall, causes overgrowths of a coarse nodular nature which soon disintegrate. It appears to be a more serious enemy to the sugar beet than crown-gall, and is one to be greatly feared should it become generally disseminated. We have called it tuberculosis of the beet, and have designated the yellow organism causing it *Bacterium beticolum* n. sp. (p. 194).

PLATES.

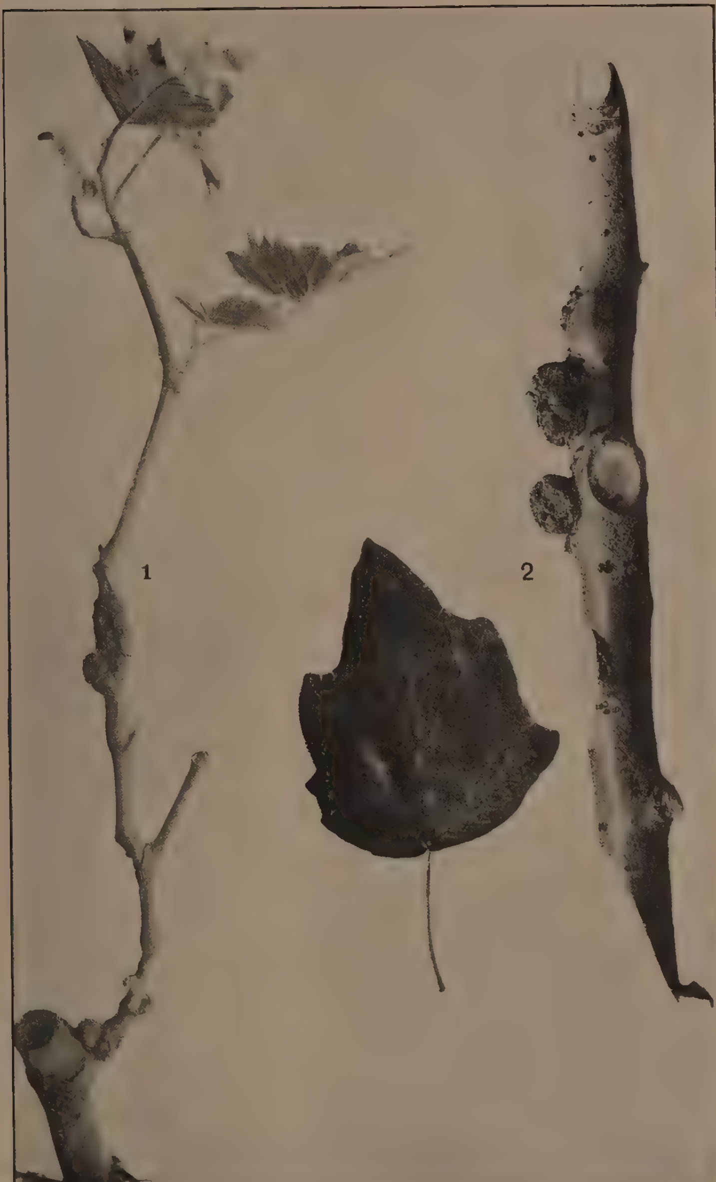
213 201

(1) Daisy on daisy. Natural size. Inoculated Dec. 13, 1906. Time: 2 months 10 days.
(2) Daisy on daisy. Three-fourths natural size. Inoculated Dec. 13, 1906. Time: 7 months.

(1) Peach on rose; inoculated Jan. 15, 1908. Time: 3 months.
(2) Apple on apple. Galls at x, x. Time: 2 months.
(3) Hop on tomato; inoculated Nov. 21, 1908. Time: 2 months 26 days.
(4) Chestnut on sugar beet; inoculated Nov. 13, 1908. Time: 33 days.
(5 a, b) Daisy on potato; inoculated Mar. 27, 1907. Time: 26 days.
(6) Rose on sugar beet; inoculated Dec. 3, 1908. Time: 19 days.

Top: At right (B and D): Daisy on oleander; inoculated Mar. 12, 1908. Time: 6 months 9 days.
At left: Natural gall on oleander from California.
Bottom: Hard gall of apple on daisy; inoculations of Nov. 9 and 18, 1908. Time: 8 months.

(1) Nematode gall on sugar beet, from Chino, Cal., 1909.
(2) Daisy on red radish; inoculated Apr. 26, 1907. Time: 3 months.



(1) Daisy on grape; inoculated Apr. 3, 1907. Time: 2 months 24 days.
(2) Daisy on gray poplar; inoculated June 9, 1908. Time: 6 months 15 days.

(1) Daisy on peach; about two-thirds natural size; inoculated Mar. 11, 1907. Time: 10 months 18 days.

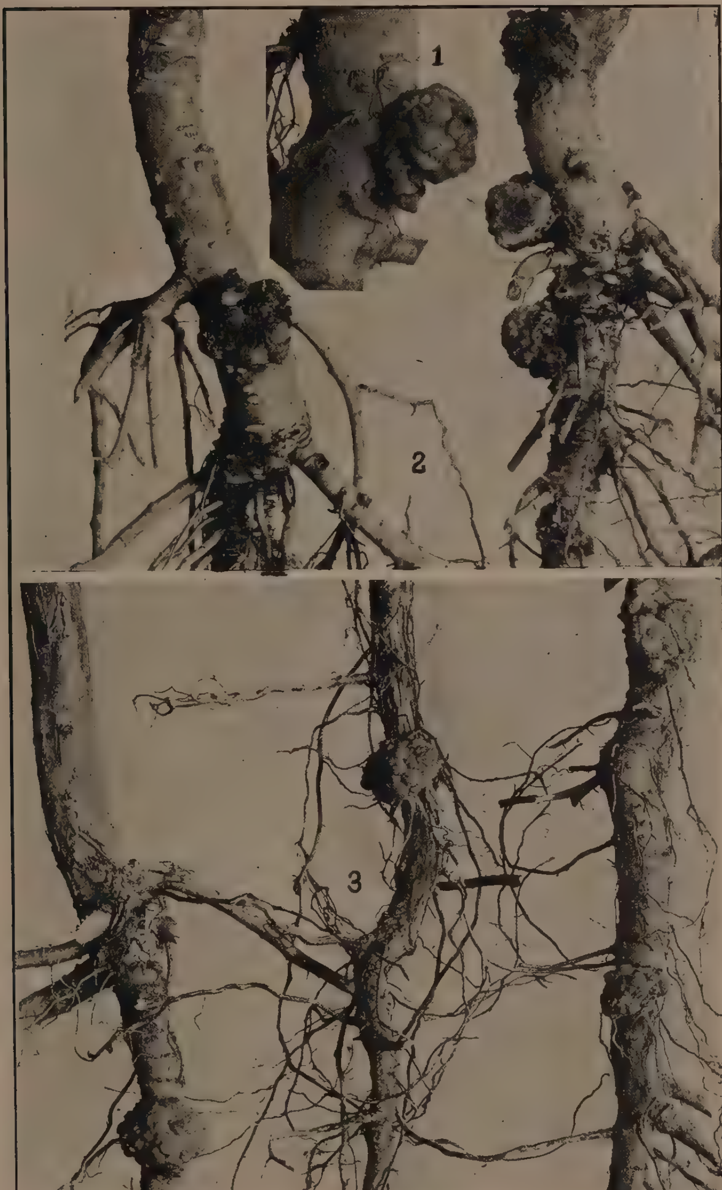
(2) Peach on sugar beet; inoculated Mar. 11, 1908. Time: 54 days.

(1) Daisy on carnation; inoculated Mar. 2, 1907. Time: 6 months 16 days.
(2) Rose on daisy; inoculated Mar. 21, 1909. Time: 5 months 23 days.
(3) Alfalfa on sugar beet; inoculated June 14, 1909. Time: 2 months 9 days.

Daisy on sugar beet. Both plants from same series. Pure-culture inoculations of Dec. 4, 1909. Time: 4 months.

(1) Daisy on hop; inoculated Apr. 10, 1907. Time: 3 months 15 days.
(2) Daisy on cut surface of raw turnip in covered Petri dish in laboratory.
(3) Grape on almond; inoculated June 28, 1910. Time: 31 days.

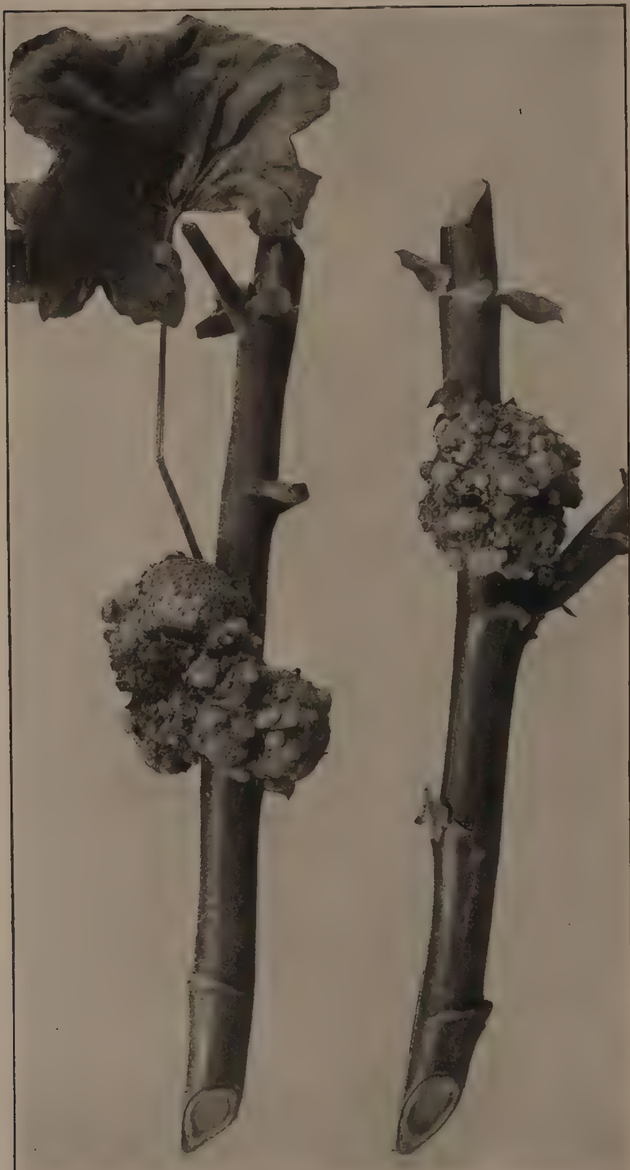
(1) Grape on grape; inoculated Aug. 31, 1909. Time: 43 days.
(2) Grape on daisy; inoculated Aug. 31, 1909. Time: 4 months 19 days.
(3) Grape on daisy at the crown; from same series as fig. 2. Time: 7 months 19 days.



(1) Peach on peach; inoculated Dec. 5, 1907. Time: 41 days.
(2) Daisy on peach; inoculated Apr. 6, 1907. Time: 3 months 6 days.
(3) Peach on peach, second series; inoculated Jan. 13, 1908. Time: 50 days.

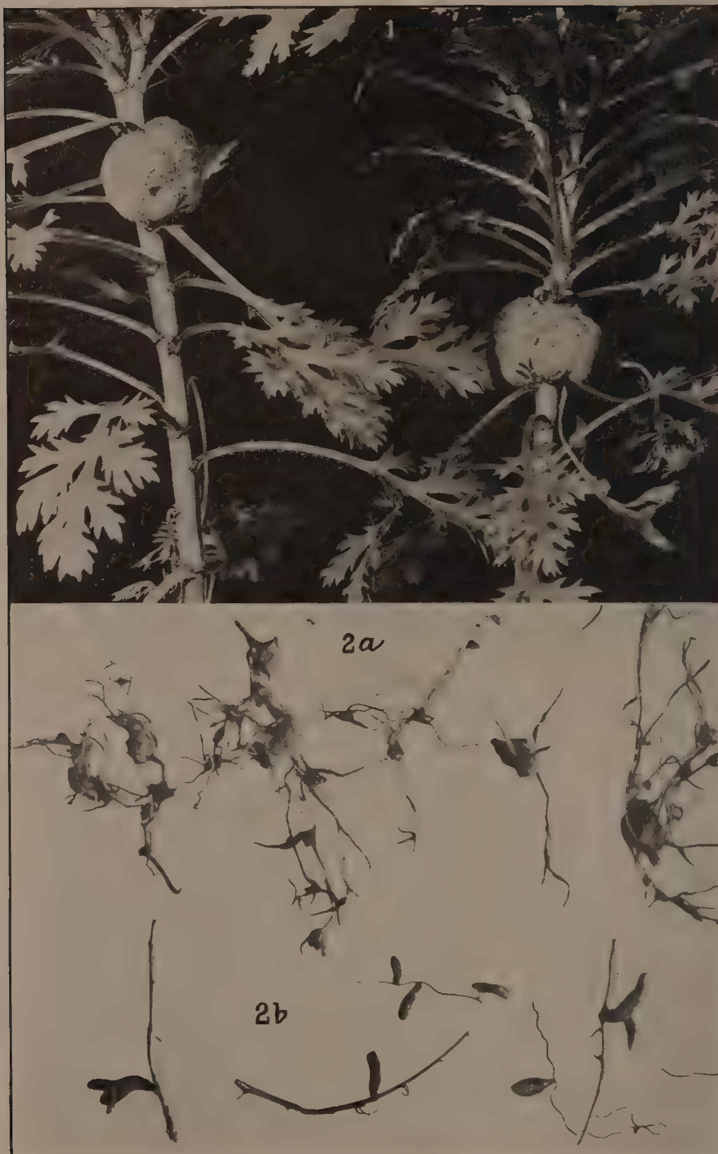
(1) Hop on sugar beet; inoculated Apr. 17, 1908. Time: 31 days.
(2) Soft gall of peach producing hard gall on apple. Three-fourths natural size. Time: 2 years.

Peach on daisy: (1) Inoculated Dec. 4, 1907. Time: 5 months 12 days. (2) Inoculated Feb. 3, 1908, with colonies plated from one of the growths shown in fig. 1. Time: 4 months.



Peach on geranium (Pelargonium). Slightly under natural size. Inoculated Oct. 13, 1908. Time: 3 months.

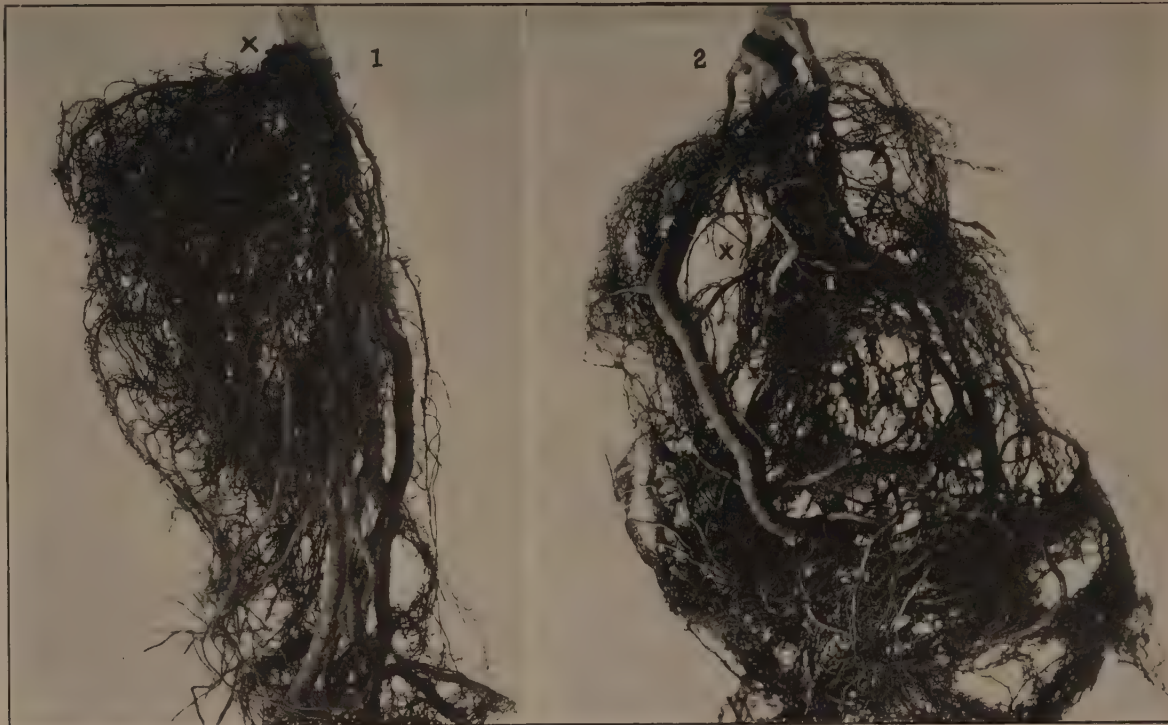
(1) Apple on daisy; inoculated Oct. 22, 1908. Time: 10 months.
(2) Hop on almond (one gall on crown, one on stem above crown); inoculated Apr. 16, 1909. Time: 7 months.



(1) Chestnut on daisy; less than natural size; inoculated Nov. 13, 1908. Time: 4 months 10 days.

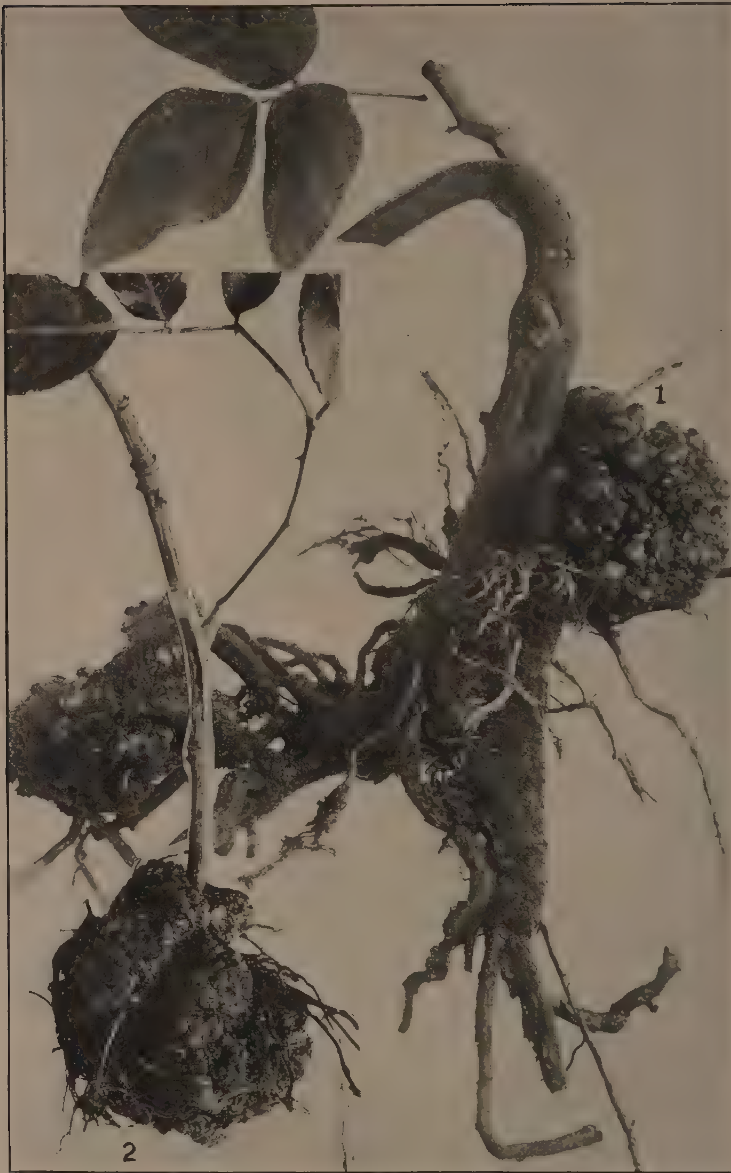
(2) a, Alfalfa on alfalfa; inoculated Sept. 7, 1909. Time: 2 months 25 days. b, Ordinary nitrogen-fixing nodules of alfalfa, introduced for comparison.

Hairy-root of apple on sugar beet: (1, 2) From one series of inoculations; the normal lateral roots are shown at x, x; inoculated Dec. 22, 1908. Time: 3 months 19 days. (3) Enlarged from another series; inoculated Nov. 11, 1909. Time: 4 months 27 days.

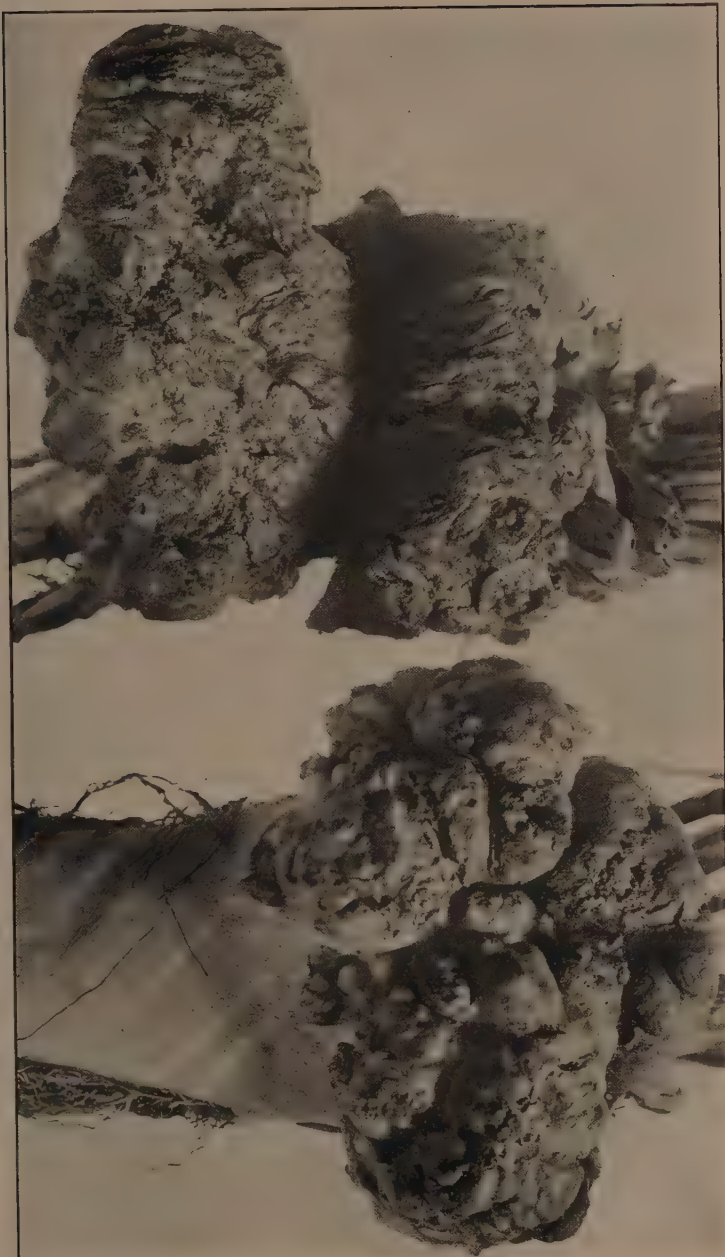


Apple hairy-root inoculated on young apple trees, both photographed after several months in alcohol: (1) Hard gall at x. (2) Typical fleshy roots at right of x. Inoculations of Apr. 5, 1909. Time: 4 months 8 days.

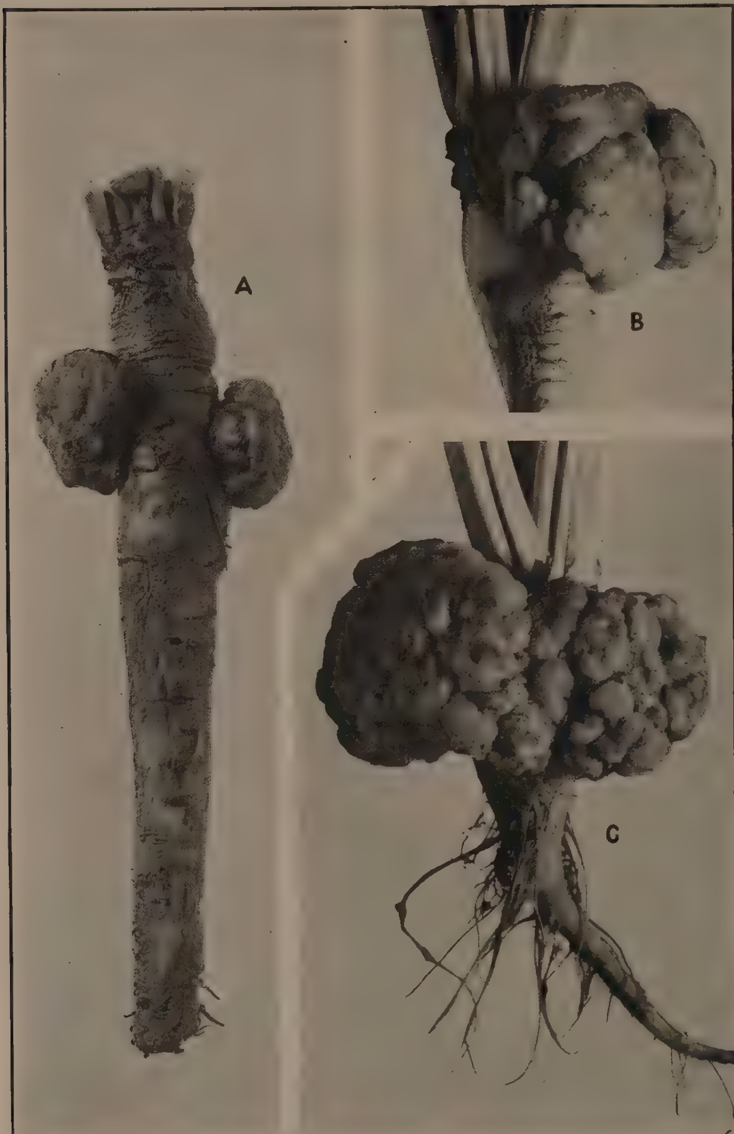
Hairy-root of apple on sugar beet. Two sides of the same beet enlarged twice to show small galls with clusters of roots originating therefrom. Inoculated Nov. 11, 1909. Time: 4 months 27 days.



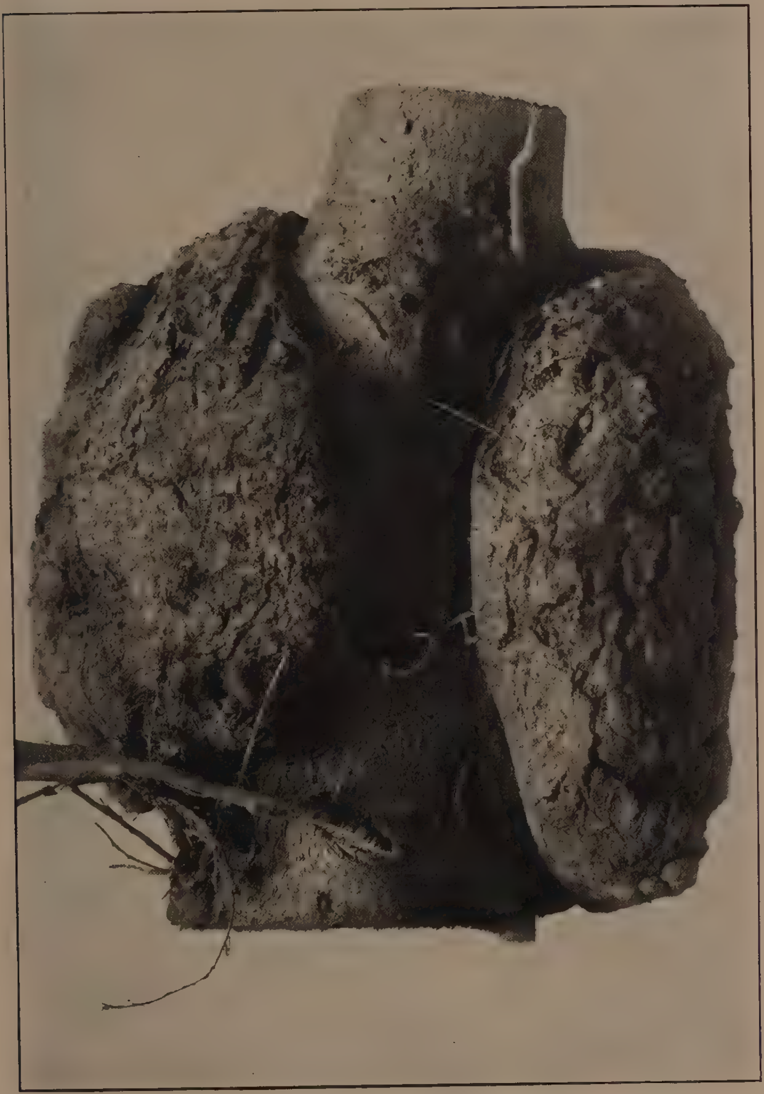
(1) Stizolobium pruriens S. P. I. No. 21300. A nematode infection occurring in the hothouse and supposed at first to be crown-gall; young gall on crown, old decaying gall on root at left.
(2) Natural crown-gall infection of young rose, from a hothouse in New Jersey.



Hop on sugar beets. About four-fifths natural size. Inoculations of Mar. 7, 1910, from twenty-sixth subculture. Time: 2 months.



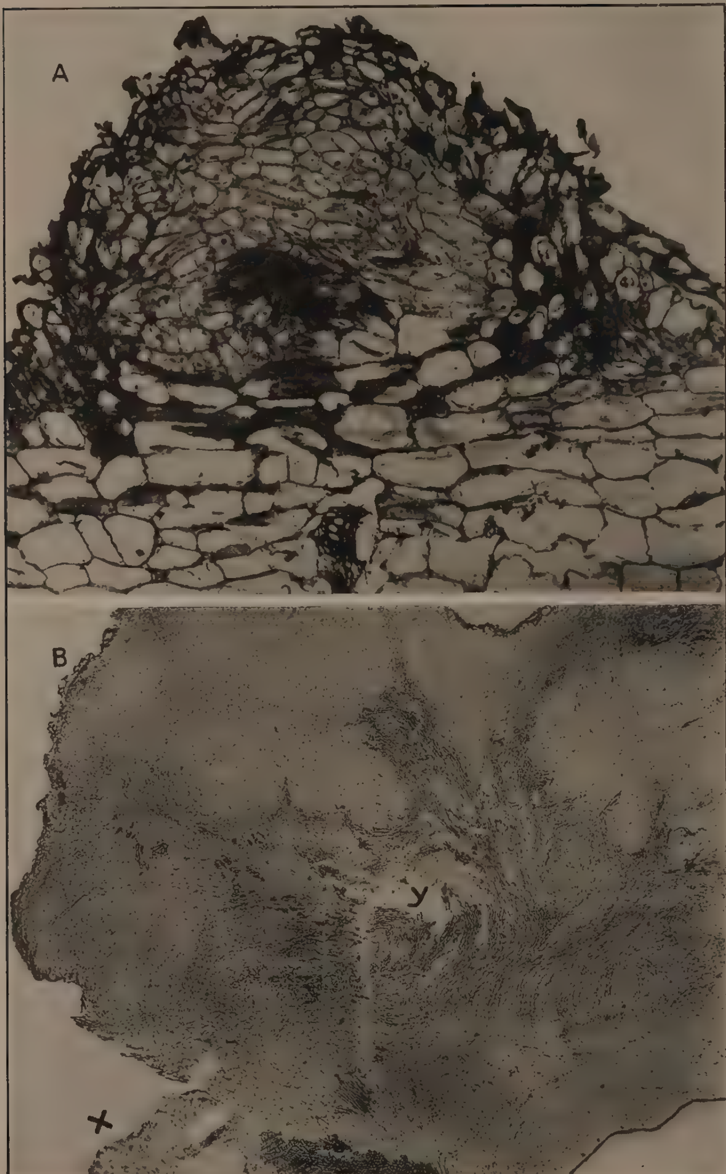
(A) Daisy on salsify. Pure-culture inoculations of Feb. 27, 1908, at the points where the galls developed. Time: 2 months 9 days.
(B, C) Poplar on sugar beet. Pure-culture inoculations of June 4, 1910. Time: 31 days.



Crown-gall on white poplar from Newport, R. I. Three-fourths natural size. Stem above the gall much dwarfed. Except that part here shown, the gall entirely surrounded the stem.

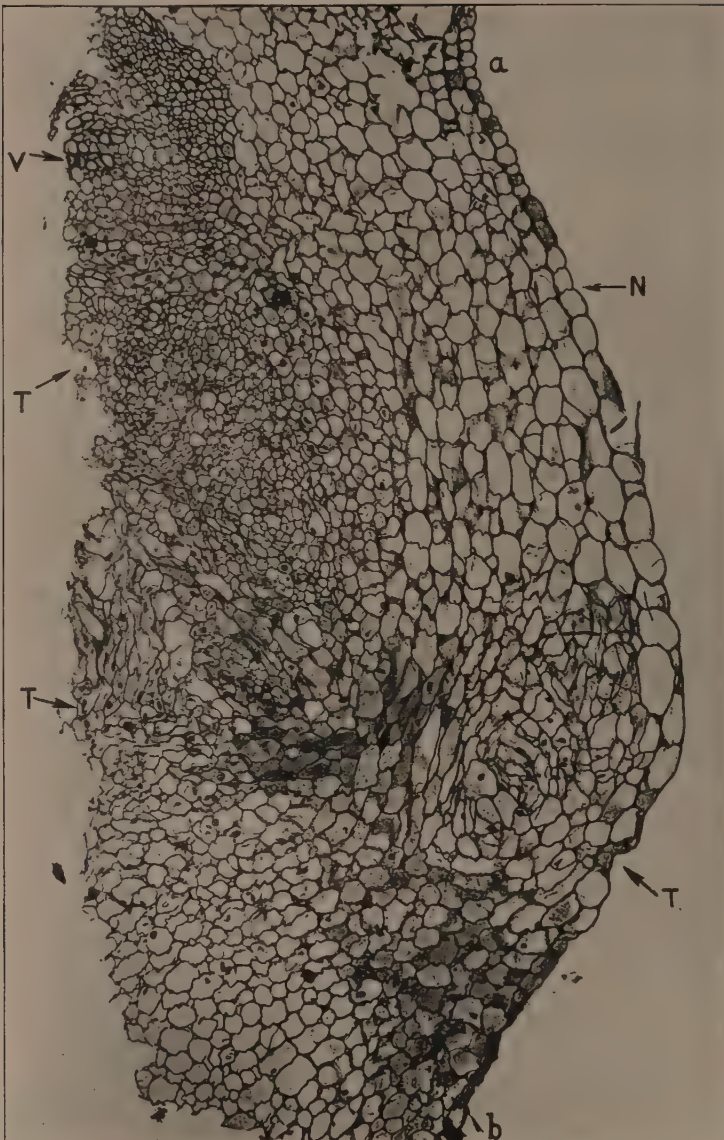
(A) Arbutus on sugar beet. Tumor partly decayed. About three-fourths natural size. Inoculated Nov. 8, 1909. Time: 7 months.
(B) Grape on sugar beet. Inoculated May 7, 1910. Slightly less than natural size. Tumor actively proliferating. Time: 47 days.
(C) Flats poplar developing on immature grape stem. Inoculated June 4, 1910. Every one of the punctures gave a tumor. Time: 1 month 14 days.

Bacterium tumefaciens on culture media: (a) Daisy organism; agar plate from bouillon; incubated 4 days at 22 to 25° C. (b) Daisy organism from a tumor; agar plate 8 days old. (c) Gall organism from a peach gall; agar plate 14 days old. (d) Hard gall of apple on agar poured plate at end of 5 days after being used for inoculations. The large colony is an intruder. (e) Same as a. (f) Needle stroke of daisy organism on slant agar, photographed after some days. (g) Old tube of sterile milk. (h) Similar tube inoculated 2 months with daisy organism, showing the pellicle formed and slow separation of whey from casein which remains undigested and fluid.



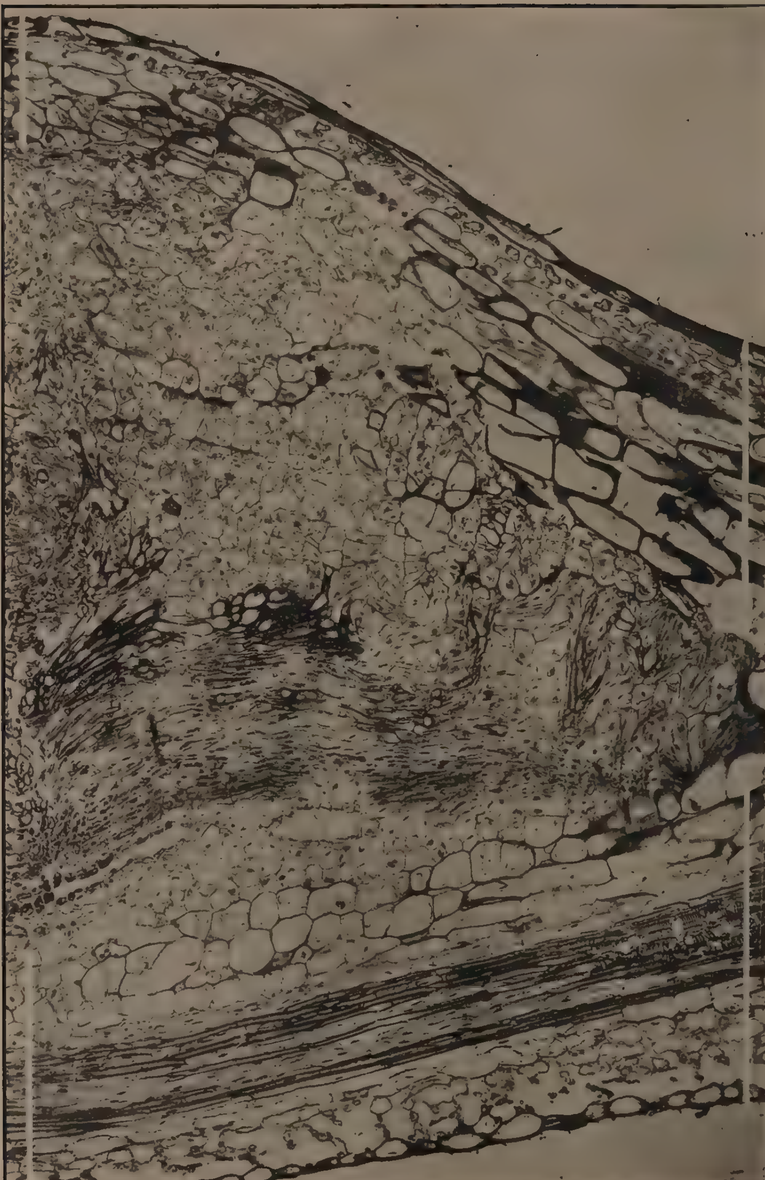
(A) Photomicrograph of section through a very young daisy gall on rib of daisy leaf. The most of the cells of the leaf rib are not yet involved in the abnormal growth.
(B) Photomicrograph of section of small daisy gall, showing supporting stroma and abnormal conductive tissue at y. A small portion of the nearly normal tissue is shown at x.

Photomicrograph of section through a rapidly proliferating gall on tobacco. The centers of most active proliferation may be seen crowding the older parenchyma cells out of place. Here and there (x, x, x) may be seen small groups of abnormal vascular bundles. Margin at top. Toward the left at top is nearly unchanged parenchyma.



Cross section of a daisy stem 10 days after inoculation with Bacterium tumefaciens: N, Normal epidermis and cortical parenchyma. V, Vascular bundles nearly unchanged. T, T, T, Rapidly proliferating tumor tissue at a distance of 1 or 2 mm. from the needle puncture. The tissue is pushed up over this hyperplasia from a to b, indicating location of the future gall.

Cross section of outer part of a tobacco stem. The lower half of the plate shows normal cortical parenchyma; the upper (outer) half, rapidly proliferating small-celled tumor tissue resulting from an inoculation. In the upper right-hand corner (in cross section) is a recently developed vascular bundle.



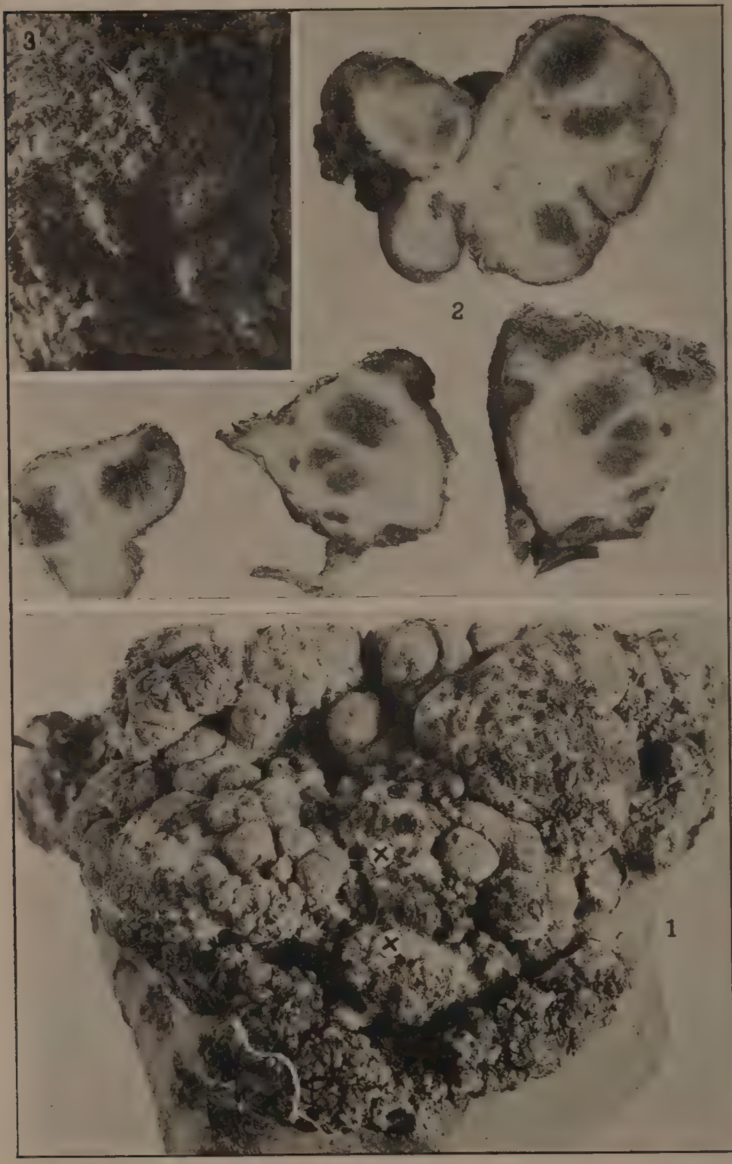
Radial section through a daisy petiole showing the internal origin of a small metastatic tumor. The normal tissues are bracketed, the epidermis is not yet ruptured, and the tumor includes all kinds of tissues peculiar to the petiole.

(A) Limb of Spitzenberg apple from Oregon attacked by a hard gall. Introduced to show a secondary infection by the pear-blight organism (B. amylovorus) radiating from the gall. x, y, Blighting areas covered by the bacterial exudate.

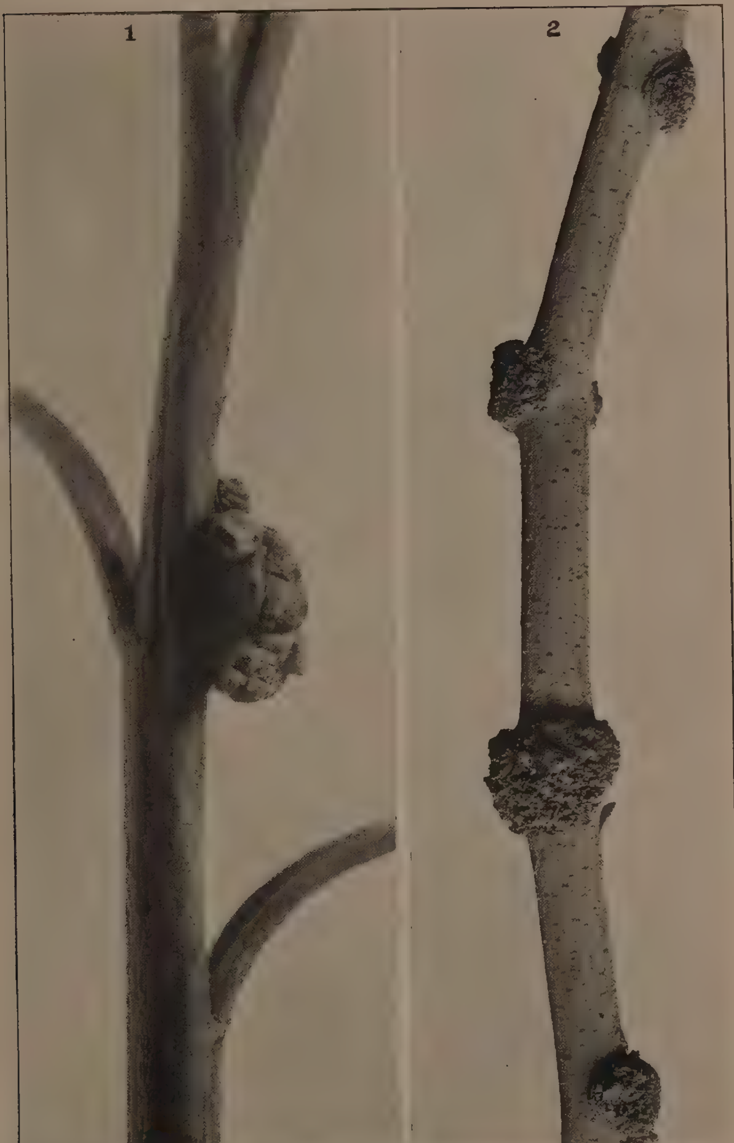
(B) Destructive galls on blackberry received from Prof. L. R. Jones, Madison, Wis. Autumn of 1910.

Photomicrograph of cross section of a daisy stem including a portion of a gall. Introduced to show centers of rapid proliferation. At the right is a portion of the normal stem—wood, bark, epidermis.

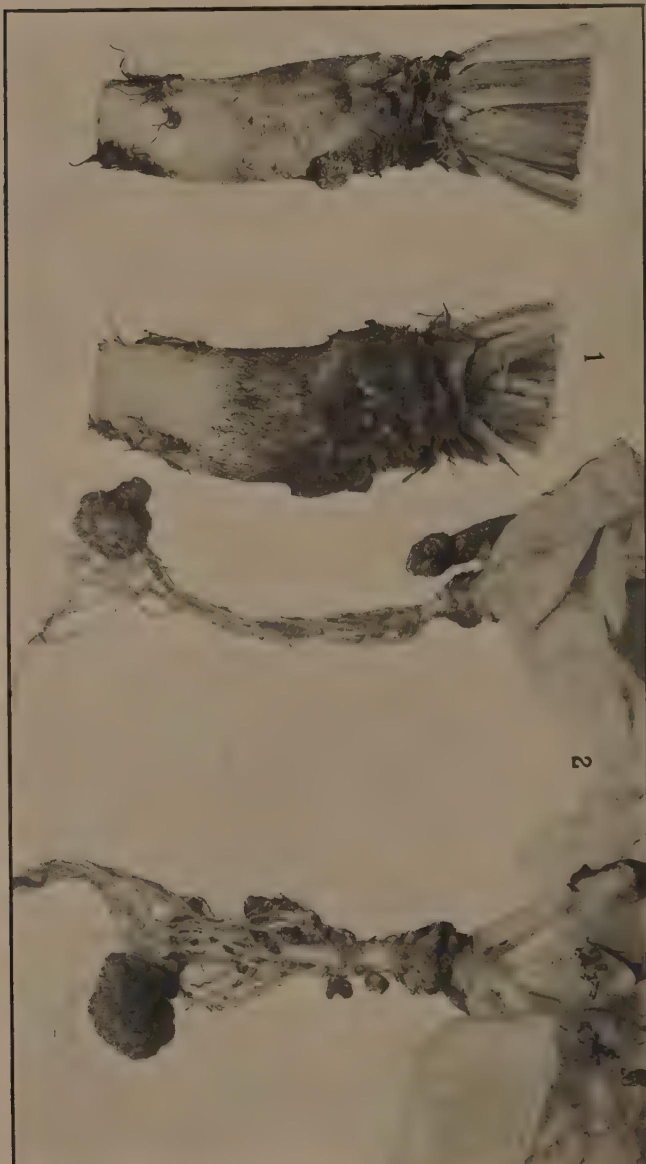
Crown-galls on Brassica due to inoculation: (B) Cabbage. (C) Collard. (A) Enlarged view of C, showing clusters of roots (hairy-root) growing out of the lower half of the gall; year, 1910; time from needle pricks to photograph: 3 months. (D) Hairy-root of apple on quince. Time: 19 months.



(1) Sugar beet from Colorado, showing bacterial tubercles distinct from crown-galls, attacked by fungi, at x, x.
(2) Sections of some of the upper nodules showing the central brownish water-soaked bacterial areas surrounded by white flesh.
(3) The surface of one of the nodules much magnified, to show the small central rifts in the tissue, referred to in the text.



(1) Gall on Salix babylonica induced by needle prick introducing pure subculture of Bacterium tumefaciens plated from a South African willow gall. Enlarged.
(2) Galled quince stem from Dr. Trabut in Algeria for comparison with Plate XXXIII, fig. D.



(1) Beet on beet; inoculations of December, 1910. Time: 44 and 52 days.
(2) Hothouse lettuce, Maryland, January, 1911. Badly dwarfed.

INDEX.

213

213

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[Continued from page 2 of cover.